IN-VIVO BONE-BORNE EXPANSION PROTOCOLS WITH PIEZOELECTRIC SUTURAL CORTICOTOMY

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IN-VIVO BONE-BORNE EXPANSION PROTOCOLS WITH PIEZOELECTRIC SUTURAL CORTICOTOMY

ABSTRACT

Objectives: This study consisted of two phases. In phase one, the effect of accelerated bone-borne expansion protocols on sutural separation and new sutural bone formation were evaluated using micro-computed tomography, histomorphometry, and immunohistochemistry. The optimum instant sutural expansion possible without disruption of bone remodeling and formation was also determined. In phase two, the effects of piezoelectric sutural corticotomy on the amount of sutural separation and new suture bone formation with accelerated bone-borne sutural expansion were studied. Differences between continuous and discontinuous sutural corticotomy was also explored. **Materials and Methods**: In this study, sixteen 20 to 24-week old New Zealand white rabbits were randomly divided into 4 experimental groups for phase one. For phase two, 14 rabbits were randomly divided into 3 experimental groups. Modified hyrax expanders were placed across the midsagittal sutures of the rabbits and secured with miniscrew implants located bilaterally in the frontal bone. In phase one, the hyrax appliances were activated as follows: Group 1 (control), 0.5 mm/day expansion for 12 days; group 2, 1 mm instant expansion followed by 0.5 mm/day for 10 days; group 3, 2.5 mm instant expansion followed by 0.5 mm/day for 7 days, and group 4, 4 mm instant expansion followed by 0.5 mm/day for 4 days. In the phase two, the groups were as follows: Group 1, accelerated sutural expansion; group 2, accelerated sutural expansion with continuous corticotomy; and group 3, accelerated sutural expansion with discontinuous corticotomy. All sutural corticotomies were performed using a piezoelectric instrument prior to expander application under anesthesia. The hyrax expanders were activated 2.5 mm instant expansion followed by 0.5 mm/day for 7 days. After 6 weeks of retention, bone volume fraction, sutural separation, and new bone formation were evaluated using micro-computed tomography and histomorphometry/immunohistochemistry. **Results:** In phase one, the smallest median sutural separation was observed with group 1 (3.05 mm) and the greatest with group 4 (4.57 mm). The least and most bone formation was observed with group 4 (55.82%) and group 3 (66.93%), respectively. A significant correlation ($r=0.95$, $p<0.01$) was observed between the amount of instant expansion and sutural separation. In phase two, ranking of median sutural separation was as follows: Group 1 (3.97 mm), group 3 (4.97 mm) and group 2 (5.58 mm). The least and most bone formation were observed with groups 1 (66.93%) and 2 (76.25%), respectively. Spearman's correlation showed strong, positive and significant correlation ($r= 0.881$, $p<0.01$) between the new sutural bone formation and amount of sutural separation. **Conclusion:** The sutural bone formation corresponded with the amount of instant expansion to a critical point beyond which the ability of the suture to remodel was disrupted. The protocol involving 2.5 mm instant expansion was the optimal for accelerated sutural expansion. When 4 mm instant expansion was employed, new suture bone formation was decreased. In addition, piezoelectric sutural corticotomies increased sutural separation and promoted new sutural bone formation. Continuous corticotomy gave better results than discontinuous corticotomy.

Keywords: Orthodontics, sutural separation, sutural bone formation, piezoelectric corticotomy.

***IN-VIVO* BONE-BORNE EXPANSION PROTOCOLS WITH PIEZOELECTRIC SUTURAL CORTICOTOMY**

ABSTRAK

Objektif: Kajian ini terdiri daripada dua fasa. Dalam fasa pertama, kesan protokol pengembangan tanggungan tulang yang dipercepatkan terhadap pemisahan sutur dan pembentukan tulang sutur baharu dinilai menggunakan tomografi berpengiraan-mikro, histomorphometri, dan imunohistokimia. Kemungkinan pengembangan segera sutur yang optimum tanpa terganggunya pemodelan semula dan pembentukan tulang juga dipastikan. Dalam fasa kedua, kesan piezoelektrik pembelahan sutur terhadap jumlah pemisahan sutur dan pembentukan tulang sutur yang baharu dengan pengembangan sutur yang dipercepatkan menggunakan tanggungan tulang telah dikaji. Perbezaan antara pembelahan sutur bersambung dan tidak bersambung juga dikaji. **Bahan dan Kaedah:** Dalam kajian ini, 16 ekor arnab putih New Zealand yang berusia antara 20 hingga 24 minggu diasingkan secara rawak kepada empat kumpulan eksperimen bagi fasa pertama. Bagi fasa kedua, 14 ekor arnab dibahagikan secara rawak kepada tiga kumpulan eksperimen. Pengembang Hyrax yang telah diubah diletakkan merentas sutur sagital tengah arnab-arnab tersebut dan dikuatkan dengan implan skru mini yang ditanam secara dwisisi dalam tulang frontal. Dalam fasa pertama, peralatan Hyrax diaktifkan seperti berikut: Kumpulan 1 (kawal), pengembangan sebanyak 0.5 mm/hari selama 12 hari; Kumpulan 2, 1 mm pengembangan segera diikuti dengan pengembangan sebanyak 0.5 mm/hari selama 10 hari; Kumpulan 3, 2.5 mm pengembangan segera diikuti dengan pengembangan sebanyak 0.5 mm/hari selama tujuh hari; dan Kumpulan 4, 4 mm pengembangan segera diikuti dengan pengembangan sebanyak 0.5 mm/hari selama empat hari. Dalam fasa kedua, kumpulan eksperimen adalah seperti berikut: Kumpulan 1, pengembangan sutur yang dipercepatkan; Kumpulan 2, pengembangan sutur yang dipercepatkan dengan pembelahan tulang bersambung; dan Kumpulan 3, pengembangan sutur yang dipercepatkan dengan pembelahan tulang tidak bersambung. Kesemua pembelahan sutur dilakukan menggunakan alat piezoeletrik sebelum pemasangan pengembang selepas pemberian anestesia. Pengembang Hyrax diaktifkan dengan 2.5 mm pengembangan segera diikuti dengan pengembangan sebanyak 0.5 mm/hari selama 7 hari. Selepas penahanan selama 6 minggu, jumlah belahan tulang, pemisahan sutur, dan pembentukan tulang baharu dinilai menggunakan tomografi berpengiraan-mikro dan histomorphometri/imunohistokimia. **Keputusan:** Dalam fasa pertama, median pemisahan sutur terkecil dilihat pada Kumpulan 1 (3.05 mm) dan median pemisahan sutur terbesar dilihat pada Kumpulan 4 (4.5 mm). Pembentukan tulang paling sedikit dan paling banyak masing-masing dilihat pada Kumpulan 4 (55.82%) dan Kumpulan 3 (66.93%). Korelasi ketara ($r=0.95$, $p<0.01$) kelihatan antara jumlah pengembangan segera dan pemisahan sutur. Dalam fasa kedua, kedudukan median pemisahan sutur adalah seperti berikut: Kumpulan 1 (3.97 mm), Kumpulan 3 (4.97 mm), dan Kumpulan 2 (5.58 mm). Pembentukan tulang paling sedikit dan paling banyak masing-masing dilihat pada Kumpulan 1 (66.93%) dan Kumpulan 2 (76.25%). Korelasi Spearman menunjukkan korelasi yang kuat, positif dan ketara ($r= 0.881$, $p<0.01$) antara pembentukan tulang sutur yang baharu dan jumlah pemisahan sutur. **Kesimpulan:** Pembentukan tulang sutur adalah selari dengan jumlah pengembangan segera hingga titik kritikal di mana keupayaan sutur untuk pemodelan semula terganggu apabila dilampaui. Protokol yang melibatkan 2.5 mm pengembangan segera, merupakan paling optimum bagi pengembangan sutur yang dipercepatkan. Apabila 4 mm pengembangan segera dilakukan, pembentukan tulang sutur yang baharu berkurang. Selain itu, pembelahan piezoeletrik sutur meningkatkan pemisahan sutur dan menggalakkan pembentukan tulang sutur yang baharu. Pembelahan tulang bersambung menghasilkan keputusan yang lebih baik berbanding pembelahan tulang tidak bersambung.

Kata kunci: Orthodontik, pemisahan sutur, pembentukan tulang sutur, pembelahan piezoeletrik sutur.

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ALP	:	Alkaline phosphatase antibody
BMD	:	Bone mineral density
BMU	:	Basic multicellular unit
BV/TV	:	Bone volume fraction
CBCT	:	Cone-beam computed tomography
CT	:	Computed tomographic
DO	:	Distraction osteogenesis
ECM	:	Extracellular matrix
EDTA	:	Ethylenediamine tetraacetic acid
FD	:	Fractal dimension
FOV	:	Field of view
H&E	:	Hematoxylin and eosin.
IHC	:	Immunohistochemical
ME	:	Maxillary expansion
MED	:	Magnetic expansion device
Micro-CT	:	Micro-computed tomography
MRI	:	Magnetic resonance imaging
MSIs	:	Miniscrew implants
MTD	:	Maxillary transverse deficiency
OPG	:	Osteoprotegerin
OPN	:	Osteopontin antibody
PA	:	Periapical
PBS	:	Phosphate-buffered saline

PCNA	:	Proliferating cell nuclear antigen
PTH	:	Parathyroid hormone
RAP	:	Regional acceleratory phenomenon
RME	:	Rapid maxillary expansion
ROI	:	Region of interest
SAME	:	Surgically assisted maxillary expansion
SARME	:	Surgically assisted rapid maxillary expansion
SME	:	Slow maxillary expansion
SNR	:	Signal to noise ratio
Tb.N	:	Trabecular number
Tb.Sp	:	Trabecular separation
Tb.Th	:	Trabecular thickness
TRAP	:	Tartrate-resistant acid phosphatase

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background

1.1.1 Bone-borne rapid maxillary expansion

One of the most prevalent forms of skeletal dysplasia encountered in clinical dentistry is Maxillary transverse deficiency (MTD). It is usually classified based on unilateral or bilateral crossbite, and anterior crowding (Howe et al., 1983; McNamara, 2000). The etiology of MTD is multifactorial in nature and environmental, genetic, developmental as well as iatrogenic factors have been cited as contributing factors (Betts et al., 1995; Haas, 1970; Harvold et al., 1972; Odont, 2001). Patients with some craniofacial syndromes, such as Treacher-Collins and Cleidocranial dysplasia, frequently present with narrow maxillary arches. Parafunctional habits like thumb sucking and mouth breathing can lead to the development of anterior open bite and/or high palatal vaults. Approximately 23.3% of malocclusions within the primary dentition population present with MTD (Brunetto et al., 2017; Kurol & Berglund, 1992).

Rapid maxillary expansion (RME) has been promoted as the treatment of choice for the correction of MTD (Angell, 1860; Baccetti et al., 2001). RME has been indicated to correct MTD, relieve crowding, alter arch perimeter, correct posterior crossbite and adjust arch forms to facilitate non-extraction treatment plan in certain cases. Expansion from RME consists of three components: alveolar expansion, midpalatal sutural expansion, and dental tipping (Adkins et al., 1990; Garrett et al., 2008). The conventional appliance for the treatment of MTD is a tooth-borne maxillary expander (Erverdi et al., 1994; Mundstock et al., 2007; Shapiro & Kokich, 1988). However, in these appliances, expansion forces transferred to the bone through the teeth, may lead to the tipping of teeth (Smalley et al., 1988), root resorption (Erverdi et al., 1994), limited skeletal movement (Shapiro & Kokich, 1988) and poor anchorage resulting in minimum amount of sutural expansion (Parr et al., 1997). An alternative to this method is to anchor the appliance directly to the palatal surfaces of the maxilla with a miniscrew implant (bone-borne maxillary expander) (Harzer et al., 2004; Lagravère et al., 2010; Lee et al., 2010; Proffit et al., 2014). Bone-borne maxillary expanders can minimize the undesirable effects associated with the tooth-borne maxillary expanders and it directs most of the expander forces on the bone resulting in increased skeletal expansion. Several studies have evaluated the effect of using the bone-borne maxillary expander for the treatment of MTD. For example, in 2014, Lin and coworkers used cone-beam computed tomography (CBCT) to evaluate the immediate effects of bone-borne and tooth-borne maxillary expander on the dentoalveolar and transverse skeletal components in late adolescents. They reported that the bone-borne maxillary expander produced fewer dentoalveolar side effects and greater orthopedic effects compared with tooth-borne appliance. It seems crucial to incorporate bone anchorage to secure the expansion of the maxillary basal bone (Lin et al., 2014). In 2015, the effects of a bone-borne maxillary expander in comparison with a tooth-borne maxillary expander in growing patients were evaluated and compared. It has been found that bone-borne maxillary expansion can be a good substitute to the tooth-borne one, especially when the patients had reduced anchorage teeth or inhibited vertical growth patterns (Chane-Fane & Darqué, 2015; Mosleh et al., 2015; Yılmaz et al., 2015). Furthermore, they are well-tolerated and easier to use than traditional tooth-borne expanders (Garreau et al., 2016).

The efficacy of bone-borne RME is influenced by several factors including the rate of distraction or activation. Clinical activation protocols for tooth-borne expanders may not be applicable to bone-borne ones. As there is currently no consensus on conventions for bone-borne expanders, expansion protocols for these appliances warrant investigation (Carvalho Trojan et al., 2016). In 2005, it has been stated that when distraction is performed too rapidly for tooth-borne expanders, collagen fibers lose contact and no ingrowth of new bone occurs resulting in non-union or mal-union of the separated sutures. Conversely, if distraction is done too slowly, premature bone consolidation can occur and the required expansion cannot be achieved (Koudstaal et al., 2005). The quality and quantity of bone formation are therefore dependent, partially, on the rate of sutural expansion. While higher expansion rate has been associated with greater sutural separation (Koudstaal et al., 2005), the exact nature of this association, as well as the maximum instant expansion possible without disruption of suture remodeling, remains uncertain.

1.1.2 Sutural piezoelectric corticotomy

One of the undesirable effects of non-surgical maxillary expansion in adults is the generation of excessive stresses at the midpalatal suture which may lead to a varying degree of pain. To overcome this undesirable effect, tooth-borne surgically assisted RME (SARME) was suggested. Traditionally, tooth-borne SARME encompassing osteotomy of midpalatal suture and/or lateral maxillae and expansion appliances produced acceptable results in adolescents and adults (Bays & Greco, 1992; Kraut, 1984). However, this technique is associated with many complications such as buccal tilting, devitalization, and/or extrusion of the anchoring teeth, pressure necrosis of the palatal mucosa and fractures of the alveolar processes (Lehman Jr & Haas, 1990). Furthermore, conventional tooth-borne SARME should be followed by a retention period of 3 to 6 months before orthodontic treatment initiation (Glassman et al., 1984; Kraut, 1984). To avoid these problems, bone-borne SARME that allow bodily movement of maxillae with slight forces exerted on dental tissues and alveolar processes, preservation of the palatal arch configuration, widening of the paranasal sinuses and immediate multi-bracket therapy following arch expansion was advocated. In 2014, Lee and coworkers conducted a study on dry skulls of adult human to evaluate effect of bone-borne RME with and without surgical intervention on the craniofacial structures using finite element analysis. They concluded that different surgical models exhibited similar amounts of stress and displacement along the teeth, midpalatal sutures, and craniofacial sutures. They, however, suggested that midpalatal suture separation, which is minimally invasive, be performed to enhance bone-borne maxillary expansion. A further study might be required to evaluate the total treatment effect when applying the full activation of the jack screw (Lee et al., 2014).

Regardless of the surgical models used, osteotomy is among the most technique sensitive procedures in maxillofacial surgery. Osteotomies are frequently performed in proximity of delicate and vital anatomic structures, such as vestibular and lingual/palatal soft tissues which supply bone vascularization through the periosteum. In addition, bone is a hard tissue and many cutting or drilling tools used in osteotomies are relatively crude. In particular, high-speed rotary instruments are potentially injurious, due to the production of excessively high temperatures during osteotomies leading to impaired tissue regeneration and marginal osteonecrosis (Kerawala et al., 1999; Rupprecht et al., 2003).

Piezoelectric bone surgery (piezosurgery) was introduced as an alternative to rotating instruments. Piezosurgery is a bone cutting system which uses ultrasonic micro-vibrations. It is a meticulous, promising, and soft tissue sparing system which was developed in 1988 by Tomaso Vercellotti. In addition to the ease of clinical application, experimental evidence on animal models evaluating wound healing and bone formation suggest a favorable tissue response to piezosurgery in comparison to conventional bone-cutting techniques (Fiorellini, 2005; Vercellotti, 2000). Several clinical applications of piezoelectric surgery in SARME and Le Fort I osteotomy have been published. SARME has been used in Le Fort I osteotomy (from the pyriform rim to the pterygomaxillary junction) (Robiony et al., 2007); mandibular body, segmented, and sagittal split osteotomies (Landes et al., 2008a; Landes et al., 2008b); Le-Fort 1 maxillary osteotomy without the down-fracture technique (Rana et al., 2013) and maxillary cortical osteotomy from the distal surface of the maxillary tuberosity to the lateral piriform aperture (Koszowski et al., 2015). They reported that the piezoelectric osteotomy did not prolong the operation time, resulted in minimal bleeding, was minimally invasive and had advantages over the usual technique in providing direct vision, accomplishment of a precise and safe osteotomy. They concluded that it is possible to perform SARME by using a piezoelectric bone-cutting device. However, the evaluation of the effects of using piezoelectric sutural corticotomy on sutural separation and new suture bone formation with accelerated bone-borne sutural expansion has not been performed.

1.2 Aims of the study

This study had two phases: The aim of phase one was to evaluate the relationship between the amount of the instant (initial) expansion, sutural separation and new suture bone formation for the accelerated bone-borne sutural expansion. The aim of phase two was to determine the effects of piezoelectric sutural corticotomy on the amount of sutural separation and new suture bone formation with accelerated bone-borne sutural expansion. Furthermore, a novel modification of hyrax expander was designed to allow sutural expansion, which has never been used in an animal model.

1.3 Objectives of the study

The objectives of this study were:

1) To evaluate the success of a modified hyrax expander with mini-implants for the sutural expansion on the rabbit model via micro-computed tomography (micro-CT).

2) To investigate the effect of accelerated bone-borne expansion protocols on sutural separation and new suture bone formation using micro-CT and histomorphometry.
3) To identify the optimum instant sutural expansion possible without disruption of bone remodeling and formation using micro-CT and histomorphometry.
4) To evaluate the effect of piezoelectric sutural corticotomies on accelerated bone-borne sutural expansion using micro-CT and histomorphometry.
5) To evaluate the differences between continuous and discontinuous sutural corticotomy on the accelerated bone-borne sutural expansion using micro-CT and histomorphometry.

1.4 Research questions

The research questions of this study were as follows:

1) Is there any difference in sutural separation and bone formation between different bone-borne expansion protocols?
2) Is there an optimum instant sutural expansion for bone-borne sutural expansion beyond which bone remodeling and formation will be disrupted?
3) What is the effect of piezoelectric sutural corticotomies on the outcome of accelerated bone-borne sutural expansion?
4) Is sutural separation and new bone formation between continuous and discontinuous sutural piezoelectric corticotomies identical?

1.5 Significance and novelty of the study

This study evaluated the effects of the different amount of instant expansion (initial expansion) for the accelerated bone-borne maxillary expansion on the sutural separation and new suture bone formation for the first time. The use of a miniscrew implant (MSI) supported expansion appliance utilizing skeletal anchorage could increase orthopedic expansion while limiting the side effects of a tooth-borne appliance. Furthermore, it is important to determine the optimal amount of instant expansion possible without affecting the ability of the suture to remodel. Determining the optimal instant expansion will establish a database for orthodontists and researchers to avoid any mishandling of accelerated bone-borne maxillary expansion. The findings of this study added scientific information about increasing the skeletal effects of accelerated bone-borne maxillary expansion without affecting the sutural bone remodeling. In addition, this study aimed to provide a new expansion protocol, to increase the amount of new suture bone formation which might reduce post-surgical retention period, and could be applied in cases suffering from severe MTD. Furthermore, this study yielded useful information for the refinement SARME procedures. A larger amount of initial expansion may be feasible at the time of surgery under anesthesia. This could be coupled with a shorter period of gradual expansion post-surgery reducing total treatment time.

Second phase of the study focused on the effects of performing different techniques of the piezoelectric sutural corticotomies on the amount of sutural separation and new suture bone formation were studied. A novel technique to enhance the outcome of accelerated bone-borne sutural expansion was used, which is very important for the patients with advanced MTD.

1.6 Organization of the thesis

After clarifying background of the current study, our aims, and research questions, this manuscript will review available literary research context in the following chapter. Three broad parts of literature have been explored in chapter two which are: maxillary transverse deficiency, treatment options for maxillary transverse deficiency, and methods of bone tissue analysis. It also presents the possible effects of instant expansion together with sutural corticotomy. Chapter Three briefly elaborates and explains the design of study, sample characteristics, methodology, process of data collection, and data analysis. Chapter Four will enlighten the salient findings of micro-CT, histomorphometric, and immunohistochemical analysis for sutural separation and new suture bone formation after the application of different amount of instant expansion and different sutural corticotomy techniques. Chapter Five discusses the results in scientific way to reach the findings. Lastly, Chapter Six concludes the thesis with recommendations for future works.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This section describes the current literature about the MTD in growing and non-growing individuals and the current options for transverse corrections including the accelerated bone-borne sutural expansion and the surgical techniques. This is followed by a review of a minimal invasive technique for the corticotomy using piezoelectric surgery and a brief overview of the miniscrew implant and its development as a skeletal anchorage for the maxillary expansion followed by a review of our current understanding of the cranial suture and the response of sutures to stress application. Then bone remodeling in cranial bone and during the sutural expansion is reviewed. Afterwards, the current methods for bone tissue analysis including the micro-CT, histology, and immunohistochemistry methods are discussed. Finally, the selection of a rabbit animal model for sutural expansion is reviewed.

2.2 Bone expansion

In craniofacial application, the bone expansion procedures can be utilized in extra oral craniofacial bone distraction and intra oral palatal expansion. Typically, craniofacial expansion is achieved using distractor osteogenesis procedures. In 1905, Codivilla introduced distraction osteogenesis (DO) (Codivilla, 1905). In 1990, Ilizarov reported the use of DO in orthopedic surgeries (Ilizarov, 1990). The mechanism of DO is based on use of a distractor for gradual administration of distraction forces on osteotomized bone segments. The distractor is an activatable device which can mechanically create separation two bony segments apart to which the device has been attached. New trabecular growth is initiated between the two segments of bone which subsequently results in bony mineralization, this phenomenon leads to the lengthening of osteotomized bone. Optimal distraction rate for the DO is considered 1.0-1.2 mm/day (Boccaccio et al., 2007; Ilizarov, 1989). Palatal expansion is achieved using several types of appliances including maxillary expander which is also a form of distractor. This type of bone expansion involves the expansion of sutural area which differs from the expansion of long bones in other parts of the body (Ilizarov, 1989). In clinical dentistry, expansion of the maxilla is most common in the correction of MTD.

2.3 Maxillary transverse deficiency (MTD)

One of the most common skeletal problems in the craniofacial region is the MTD, predominately mixed with simultaneous anteroposterior or vertical skeletal discrepancy (Betts et al., 1995). MTD is well-known in both syndromic and non-syndromic patients (Menon et al., 2010). Essentially the most often reported clinical manifestations are, palatally inclined maxillary teeth, dental crowding, narrow and tapered arch forms, unilateral or bilateral posterior crossbites and problems related to nasal respiration (Pereira et al., 2010). In contrast to vertical or sagittal discrepancies, MTD is difficult to diagnose extra orally. The extra oral manifestations are most commonly discrete and confined to contracted alar bases, paranasal excavating and a deep nasolabial groove. However, in many cases, the MTD exist concomitantly with vertical and sagittal discrepancy that mask the extra oral appearance of MTD.

The etiology of MTD is multifactorial, together with congenital, genetic, developmental, traumatic and/or iatrogenic explanations (Betts et al., 1995; Haas, 1970; Harvold et al., 1972; Odont, 2001). Examples of contributing factors are different syndromes, thumb and finger-sucking habits, mouth breathing during valuable growth durations, trauma or iatrogenic accidents after cleft palate repair. The incidence of MTD is reported to be 8.5 to 22 percent in growing individuals of patients having orthodontic consultations (Da Silva Filho et al., 2007). The wide range of incidence could be due to the lack of uniformity in the classification of MTD, for instance, absence of clarity with regards to the severity of dental components and the magnitude of the skeletal discrepancy (Da Silva Filho et al., 2007; Harrison & Ashby, 2001; Thilander et al., 1984). There are no differences in incidence in regard of gender or ethnicity (Allen et al., 2003) and there is no data available within the literature regarding this matter.

It is important to differentiate between dental and skeletal components of the deformity to choose the treatment modality which will achieve stable and functional outcomes (Haas, 1965). MTD can be a truly dental, a truly skeletal discrepancy or a combination of both (Bishara & Staley, 1987). In some cases, the palatal inclination of one or more posterior teeth can lead to an apparent maxillary deficiency. These maxillary deficiencies with purely dental components are, in most cases, simple orthodontic problems and do not require extensive orthodontic or surgical treatment. Bishara and Staley suggested a thorough clinical examination of MTD that accounts the magnitude of the transverse discrepancy between maxilla and mandible, the quantity of teeth involved and the preliminary angulation of the maxillary molars and premolars. A transverse discrepancy exceeding 4 mm and/ or buccally inclined maxillary molars and premolars indicate a true skeletal MTD (Bishara & Staley, 1987). There are a couple of indices for evaluating transverse dental deficiencies on study models, for example, Pont´s Index, Korkhaus Index, and Howe´s evaluation. However, these indices cannot be utilized to examine the extent of a skeletal discrepancy (Dause et al., 2010; Howes, 1947). The treatment planning can be difficult because of most cases of MTD include a mixture of dental and skeletal components. Ricketts (1998) and Grummons and Ricketts (2004) proposed the use of frontal cephalometric evaluation to distinguish between discrepancies in the widths of the dental arch, alveolar arch and skeletal base (Grummons & Ricketts, 2004; Ricketts, 1998). This analysis attempts to stratify skeletal MTD into different maxillomandibular combinations such as narrow or normal maxilla and normal or wide mandible, with a purpose to assess the severity of the deficiency (Ricketts, 1981). MTD in patients exhibiting a narrow maxilla and wide mandible was likely to be the most challenging to be corrected and the most susceptible to relapse.

Skeletal MTD may also be divided into two categories; relative and true. Relative MTD implies that a transverse discrepancy exists clinically, but is brought on by a sagittal discrepancy between the jaws, i.e. In a relative MTD, no transverse deficiency exists when the dental models were occluded in a class I relation. This phenomenon usually occurs in Angle Class III skeletal malocclusions. True MTD implies a real transverse maxillary insufficiency. Clinically there may or may not be a posterior crossbite. In contrast to relative MTD, true MTD suggests a uni- or bilateral posterior crossbite when the dental models were occluded in a class I relation. True MTD is most of the time accompanied with skeletal open bite and class II malocclusions. Although relative MTD can also be treated with midpalatal suture opening, relative MTD require no orthopedic or surgical transverse growth (Jacobs et al., 1980). In such cases, conventional orthodontics, with or without extractions is enough to correct the transverse discrepancy. In the surgical treatment of skeletal sagittal anomalies, relative MTD shall be corrected with the aid of the next sagittal displacement.

True MTD, nonetheless, requires the separation of maxillae by opening the midpalatal suture to normalize the transverse deficiency and is not able to be corrected by conventional orthodontics treatment alone (Menon et al., 2010; Vanarsdall & White Jr, 1994). As soon as the diagnosis has been made and a necessity for enlargement is ascertained, different explanations must be addressed, such as the magnitude of the transverse discrepancy, the age of patient, whether the expansion must be executed orthopedically and/or by using surgical intervention and to which extent the suture can withstand the expansion without disturbing it's potential to remodel.

2.3.1 Maxillary transverse deficiency in growing individuals

The conception of correcting MTD in growing individuals by midpalatal suture opening and a separation of the maxilla were widely employed through past century (Figure 2.1), however, the first published work was by Angell in 1860 using a jack screw appliance (Angell, 1860). An appliance consisting of two contra-rotating screws was placed in a young girl at an age of 14 years. The screws were threaded left and right and placed against the posterior maxillary teeth. According to Angell, correction of the narrow arch should be done in two weeks through separation of the maxilla along the midpalatal suture. Unfortunately, these in charge for the most influential dental journals and the scientific establishment could no longer see beyond the barriers of approved science and believed that the process used to be both impossible or too detrimental to be used and Angell's report used to be revised.

In 1893, Goddard confirmed that an appliance connected only to the maxillary first molar and premolar might separate the maxilla into halves with a view to relieving dental irregularities caused by a constricted upper jaw. The irregularity of teeth might be treated within an extra easy manner (Goddard, 1893). By the end of the 1920´s, the sensible notion of development gained popularity amongst Orthodontists, according to which, if the teeth were gently moved into their appropriate positions, the bone would develop to support them. The increase in the dental arch width after conventional orthodontics would result in the development of widening within the nasal passage. With the acceptance of this notion, maxillary expansion was practically abandoned.

However, Korkhaus and Haas reintroduced the concept in the early 1960´s as RME and confirmed its effectiveness in adjusting true and relative MTD in developing and non- skeletally mature patients (Haas, 1961; Korkhaus, 1959). Haas documented six primary indications for the application of RME: Patients having actual and relative MTD, patients with nasal stenosis, patients suffering from class III malocclusion, patients who had mature clefts, patients with anteroposterior maxillary deficiency and patient who had arch length issues. The appliance consisted of orthodontic bands around the first premolars and the first permanent maxillary molars or the deciduous first molars and joined with soldered lingual and buccal bars. The jackscrew was positioned in the center of the midpalatal suture and hooked up to the lingual bars with an acrylic base plate.

In 1965, Zimring and Isaacson used heavy orthopedic forces, up to 45 N, to distract apart the two bones at midpalatal suture (Zimring & Isaacson, 1965). These forces were not constrained to the maxilla and the midpalatal suture, therefore, also affected adjacent structures, directly or indirectly (Bell, 1982). To make sure enough separation within the midpalatal suture was achieved, the separation was once documented with an occlusal radiograph and the development of an inter-incisal diastema. Haas documented 10 scientific clinical cases with skeletal alterations after RME in both transverse, vertical and anteroposterior dimensions (Haas, 1970). Krebs in 1964 supported these findings and in implant experiences with a mean age of 7 years, confirmed stable long-term expansion within the maxillary base and nasal cavity (Krebs, 1964). Thorne and Hugo in 1960 found that nasal width between 0.4 mm to 5.7 mm, exhibited normal expansion of 1.7 mm, and noted that the effects would be lost without any retention plan (Thorne & Hugo, 1960). The primary discovering used to be nevertheless, that the ideal timing for expansion was once before and throughout the development of spurt interval (Haas, 1970; Proffit et al., 2014). Once the age of skeletal maturity has reached, only use of RME can help in attaining stable widening of the maxilla (Proffit et al., 2014). Skeletal maturity was once centered on anatomical reports of the maturing face and specifically the midpalatal suture and the adjacent circum-maxillary articulations (Silverstein & Quinn, 1997; Wertz, 1970). However, during an autopsy, Persson and Thilander in 1977 discovered the existence of bony junctions in the midpalatal suture in late adolescent cases and also open sections within the mid-twenties (Persson & Thilander, 1977). Melsen in 1975 concluded that growth at the midpalatal suture continues until around the age of 13–15 years and is then adapted through continuation of apposition until the age of 18 years (Melsen, 1975). The sutural development was assumed to coincide with the end of somatic growth (Isaacson & Ingram, 1964). Therefore, sutural closure diminishes the competencies to obtain a sufficiently stable skeletal expansion of maxilla.

However, the determination of skeletal maturity is crucial. The literature grants conflicting views concerning the age limit for achieving the orthopedic sutural opening of the maxilla. Timms and Vero in 1981 recommended 25 years of age as a higher limit for the orthopedic expansion (Timms & Vero, 1981); that is supported by the findings of Mossaz and coworkers (Mossaz et al., 1992). In contrast, Mommaerts observed limited orthopedic sutural opening within the maxilla of patients older than 12 years (Mommaerts, 1999). Alpern and Yurosko observed an average age change of five years for the closure of the maxillary suture in males and females (Alpern & Yurosko, 1987). All these variations are nonetheless, consistent with reports by Persson and Thilander of a wide difference in midpalatal suture ossification in various age groups (Persson & Thilander, 1977). As the skeletal growth is influenced by the craniofacial skeletal flexibility and sutural patency, the orthopaedic opening of the midpalatal suture is successful when the intervention is performed before reaching pubertal peak i.e. before 14–15 years of age (Baccetti et al., 2001; Ghoneima et al., 2011; Lione et al., 2008; Weissheimer et al., 2011).

Year	The event and name of author
1728-1859	Expansion techniques were employed by early dental practitioners (Fauchard, Bourdet, Fox, Belabarre, Robinson and White)
1860	The first work was published by Angell using a jack screw appliance
1877	Walter Coffin developed the Coffin spring for arch expansion
1888-1893	Farrar and Clark C. Godard discussed the feasibility of lateral expansion with mid palatal suture opening
Beginning of 20th century	ENT surgeons showed interest in the orthopedic expansion of maxilla
Late 1940`s	Graber advocated RME for the treatment of cleft lip and palate patients
1956	RME was reintroduced by Korkaus and Andrew Haas (Haas expander)
1968	William Biederman introduced the tooth-borne Hyrax expander
Early 1970`s	Haas started using RME extensively
1973	Cohen and Silverman introduced the bonded Hyrax expander
1975	Robert M. Ricketts introduced the Quad Helix
1980	Haas evaluated the stability of maxillary expansion achieved with RME
1999	Mommaerts introduced the Bone-borne Hyrax
2010	Wilmes and colleagues introduced the bone- and tooth-borne Hybrid Hyrax

Figure 2.1: Historical backgrounds for the correction of maxillary transverse deficiency (MTD) by sutural opening and separation of maxilla.

2.3.2 Maxillary transverse deficiency in non-growing individuals

Sufficient transverse maxillary dimensions are equally necessary for nongrowing and skeletally mature patients. Activation of expansion appliances in patients with ossified sutures can trigger a feeling of pain and/or strain, periodontal defects, root resorption, dental tipping, minimal skeletal effects (Figure 2.2) and fundamental relapse (Alpern & Yurosko, 1987; Barber & Sims, 1981; Wertz, 1970).

The crucial factor in MTD is the magnitude of the skeletal aspect. It is generally accepted that it is possible to achieve limited expansion of the maxilla without any separation of the midpalatal suture (Baydas et al., 2006; Betts et al., 1995; Silverstein & Quinn, 1997). Handelman in 1997 provided stable growth of long-time period as much as 5 mm in skeletally mature individuals without sutural opening (Handelman, 1997) and mentioned the work of Krebs, displaying that 50% of the expansion after RME in children consisted of maxillary alveolar bending (Krebs, 1959). Iseri and coworkers supported slow orthopedic expansion to overcome the resistance and reduce the side effects and the degree of relapse. The slower expansion would, according to Iseri, encourage the adaptation processes in the nasomaxillary structures and result in less tissue resistance (Işeri et al., 1998). Still, the stability is directly influenced by the maturity of sutural ossification and the long-term effects of such procedures have been questioned (Northway & Meade Jr, 1997; Shetty et al., 1994).

In non-developing and skeletally mature individuals, most orthodontists and maxillofacial surgeons currently endorse a combined surgical and orthodontic treatment technique, as a way to acquire stable and practical long-time period outcome, with minimal side effects (Barber & Sims, 1981; Krebs, 1959; Lee et al., 2014; Mommaerts, 1999; Zimring & Isaacson, 1965). Essentially the most common treatment choices for skeletally mature patients with MTD are SARME with or without segmental LeFort I osteotomies. However, the long-term results of such strategies had been questioned (Proffit et al., 1995). When compared with segmental LeFort I osteotomies and non-surgical orthopedic maxillary enlargement, SARME has been advocated to increase stability (Magnusson, 2013; Strömberg & Holm, 1995).

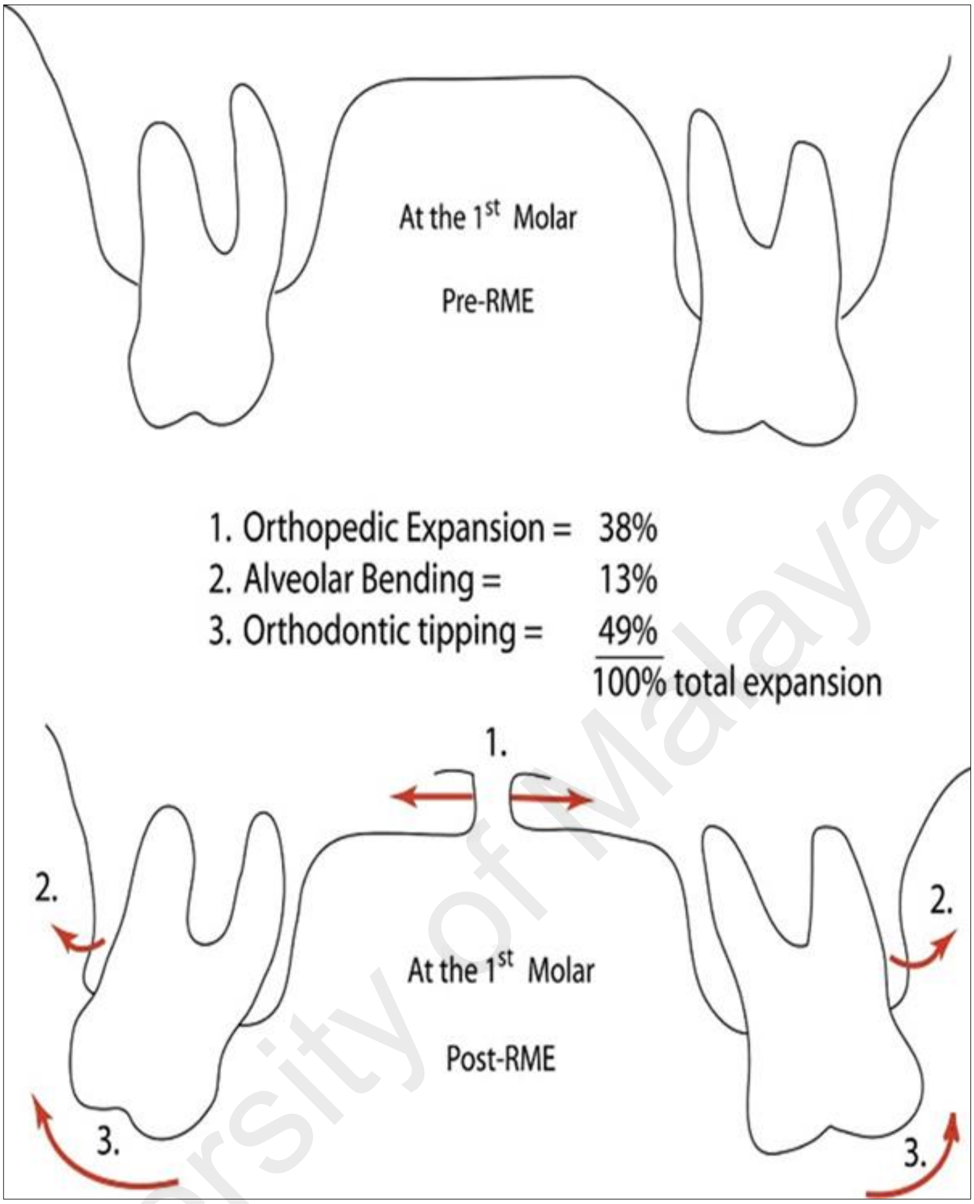


Figure 2.2: Diagram of pre- and post-tooth borne rapid maxillary expansion illustrating the 3 components of total expansion (Garrett et al., 2008).

2.4 Palatal expansion

The application of palatal expanders for the management of MTD with the aid of orthodontists commenced by the father of orthodontics, Edward Angell. His application has been generally detailed within orthodontic literature (Angell, 1860). Much of the initial work on expanders and their indications was once performed by Dr. Andrew Haas. In one of his earliest publications, Haas recommended using palatal expanders in 5 clinical instances, even though not entirely, several elements of this article released over 40 years ago are still particularly important (Haas, 1970). Interestingly, as Haas mentioned, the usage of palatal expansion as designated for Class III malocclusions is usual, and other prominent authors, such as McNamara, have relayed equivalent benefits of palatal expansion for Class III management (McNamara, 1987). Certainly, the usage of expanders as a treatment protocol remains to be as a rule used today mainly to aid the correction of crossbites, crowding, and MTD (Proffit et al., 2014).

2.4.1 Orthodontic appliances for palatal expansion

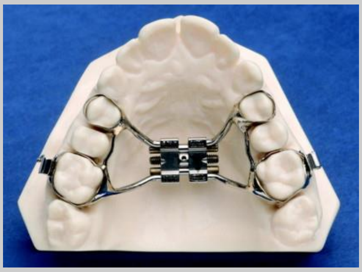
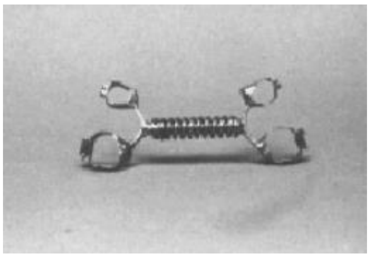
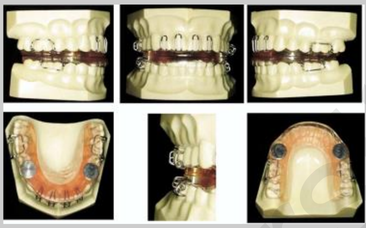
There are four types of expanders categorized according to the activation protocol. These four groups are screw-type, spring-type, magnetic, and Shape Memory Alloy appliances (Table 2.1). The screw-form expander class consists of expanders in which manual rotation through a wrench or "key" via either the clinician or patient results in widening of the jackscrew. This design has a well understood and historically original mechanical suggestion of expansion through the turning of a screw-jack. Basically, the quantity of screw rotation directly corresponds to the amount of expansion. A potential of this class entails the flexibility supplied to the practitioner to prescribe an exact quantity of expansion over a unique period. Appliances can also be tailored to suit a variety of palate sizes and shapes, reputedly confined best by means of the size and placement of the jackscrew element. However, the jackscrew appliance has many drawbacks associated to its construction. Primary issue is that upon activation of the screw, an unexpected and rapid increase in the force is produced, which has been shown to potentially result in a much less physiologic expansion of the palatal suture. In addition, this approach of expansion places a huge responsibility on the individuals to comply with expansion modality, which is a major disadvantage of this design. Within this category of appliances, Hyrax appliance designs is most commonly used (Romanyk et al., 2010).

Spring-type expanders are outlined as any appliance that functions by the way of mechanical deformation of a body. This deformation results in elastic restoration forces which can be exerted on the palate which in turn result in an expansion. This design presents many benefits; Patients do not need to manually activate the expander at certain intervals. In addition, theoretically, it applies a consistent force over an interval of time and avoids unexpected rises in force which is obvious in the screw appliances. This predictable quantity of force possibly results in greater relief for the patient following initial delivery. The amount of force produced is inversely proportional to the amount of expansion. For this reason, the more expansion produced the much less force for further expansion stays and the orthodontist may have to remove and reactivate the appliance if more expansion is needed following treatment. Patient safety can also be a bit challenging when the appliance is delivered to the patient. Despite the springs produce predictable transverse forces, any deformation of the appliance could result in undesirable forces in all different planes (Romanyk et al., 2010).

Magnetic expanders also exist and have been referred to as Magnetic expansion instruments (MED). The goal of magnetic expansion appliances is to produce steady forces of a lesser magnitude than common expansion instruments. The magnets are utilized because their directionality opposes each other, producing a repulsive and expansive force (Romanyk et al., 2010). Theoretically, this results in biologically friendly and less disturbing stimulation in the maxillary suture development, just like spring type devices. A study by Darendeliler (1994) presented the highest grade of skeletal expansion achieved in banded appliances with four magnets. A set of two magnets was positioned apically to the central and lateral incisors and the second set positioned between the second bicuspid and first molar. Despite the sample size was small, MEDs produced a slightly effective palatal enlargement, which did not depend on patient compliance (Darendeliler et al., 1994). Benefits and downsides of MEDs are like those of the spring type of appliances. However, one significant advantage of MEDs is that they're much less prone to deformation and thus have much less threat to produce undesirable and unpredictable forces in dimensions other than the transverse. Just like the spring variety, magnetic forces decrease with increasing expansion and consequently also require adjustments like its spring counterparts. This problem can be overcome by placement of magnets of greater strength, however, this will influence the patient comfort and produce physiologically lesser suture opening (Romanyk et al., 2010).

Finally, the fourth category of expanders, shape memory alloy appliances, utilize the properties of nickel titanium wires and are therefore based upon the properties of the shape memory alloy incorporated into the appliances for expansion. Orthodontists have become familiar with the properties of Ni-Ti wires. The drawbacks of a conventional spring appliance nonetheless exist with these appliances. One of the important issues existing with this expander type is that any deformation of the application could result in uncontrolled forces generated transverse to the path of expansion (Romanyk et al., 2010).

Table 2.1: Activation methods, examples, and drawbacks of the expander category.

Expander category	Examples	Drawbacks
Screw 	Hyrax, Car jack, Telescoping	Unexpected, rapid increase in force at time of activation. A huge responsibility on the individuals to comply with the expansion modality
Spring 	Coil, Wire, Minne	Any deformation of the appliance could result in undesirable force in different planes of space.
Magnetic 	Repulsion magnets	Any deformation of the appliance could result in undesirable forces generated in all different planes, but much less than spring type.
Shape memory alloy 	Coil spring, Wire spring, Screw	Uncontrolled forces generated transverse to the path of expansion.

2.4.2 Palatal expansion protocols

Expansion is commonly used for treatment of transverse discrepancies. Multiple modalities and protocols exist depending on the rate of expansion. The modalities of maxillary expansion can be classified as slow, rapid and surgically assisted (Agarwal & Mathur, 2010).

RME requires two turns per day of a jackscrew expander device, routinely an expansion rate of 0.5 mm/day is recommended. Indications for RME include transverse discrepancies of larger than 4 mm with dental compensation by way of buccally tipped maxillary molars, disruption of sutures to aid Class III correction, and moderate maxillary crowding. RME is contraindicated when there is a recession of the alveolar bone on maxillary molars or premolars, high mandibular plane angle, presence of anterior open bite, convex profile and dubious patient compliance (Agarwal & Mathur, 2010; Bishara & Staley, 1987). It is also contraindicated in mature patients past the growth spurts; nevertheless, many practitioners select this therapy modality in older patients. Clinically, activation of expander is advised once or twice a day, for two to three weeks followed by a retention interval of at the least three months. This type of activation and relatively huge force application is thought to maximize orthopedic skeletal enlargement whilst minimizing dental movements (Agarwal & Mathur, 2010). RMEs are also designed as banded or bonded appliances and can be tooth-borne, tissue-borne, or tooth and tissue borne. Some examples of rapid maxillary expanders are the Hyrax expander, Issacson expander, and Haas expander (Agarwal & Mathur, 2010).

Slow maxillary expansion (SME) is an approach during which light, somewhat continuous force levels generally in the range of 450-900 grams are applied. It's thought that the lighter forces result in much less resistance from sutural constructions, which allows more bone formation in the intermaxillary suture, however, lesser suture opening. Additionally, post- expansion stability and retention might be better with SME. Appliance designs vary extensively for SME, with some examples being the Quad helix, magnet expanders, the Coffin appliance, W-arches, and spring jets. The hyrax expander can also be used for SME, with a highest of 1 mm expansion per week applied (Agarwal & Mathur, 2010; Bassarelli et al., 2005; Mossaz-Joëlson & Mossaz, 1989).

Surgical enlargement entails both SARME and segmental maxillary surgical procedure such as a Le fort osteotomy. Certainly, this method makes it possible for expansion beyond skeletal maturation but is greatly invasive and complicated (Agarwal & Mathur, 2010; Bell & Epker, 1976).

2.4.3 Bone-borne accelerated maxillary expansion

The orthodontist's capability to treat more difficult cases without surgical intervention and surgical side effects became within the scope with the introduction of miniscrew implants or temporary anchorage devices in the 1980's. The use of miniscrew implants combined with RME is a new technique in the maxillary expansion. In this technique, the miniscrew implants are used to fixate the expansion devices to the maxillary palatal bones. A force is created when the expansion screw is turned, which then pass into to the miniscrew implants, and then to the palatal bone, which lies adjacent to the midpalatal suture. This force thereby acts to break down and open the interdigitation of the midpalatal suture between the maxillary palatal bones. These miniscrew implants are small screw-like titanium rods, ranging from 1.3-1.8 mm in diameter, and 5-11 mm in length, that anchor in the bone with roughly 75% osseointegration as it has been found in a histomorphometric evaluation on male beagle dogs (Vannet et al., 2007).

Most of the studies that evaluated this technique and their effects are summarized in the appendix A. Studies that evaluated the efficiency of using bone-borne RME with surgical osteotomy found that this technique is effective in providing palatal expansion with more skeletal effect and minimal dental tipping (Harzer et al., 2006; Iida et al., 2008). In addition, it is considered a viable treatment when there is a massive growth disturbance or disturbance expected in selected pediatric patients (Adolphs et al., 2015) and this might be due to its greater amount of skeletal effects. However, they did not evaluate the amount of sutural expansion and used 2D investigation tools for measurements. Moreover, a case report published in 2015 evaluated the use of palatal miniscrew implants for RME and mandibular setback procedure in skeletal Class III in 13 years old patient using CBCT and cephalometric (Seo et al., 2015). They reported that a bone-borne rapid expander can provide expansion with more skeletal effect and minimal dental tipping, thus improving the inclination of the posterior teeth.

A combination of both bone-borne and tooth-borne (hybrid) expander in RME was used in other studies. It has been reported that the hybrid expander is a minimally invasive expander appliance and effective for RME, especially in patients with reduced anterior dental anchorage (Akin et al., 2015; Wilmes et al., 2010). Furthermore, a randomized control trial published in 2010 studied 62 patients needing maxillary expansion (Lagravère et al., 2010). These individuals with a mean age of 14, have been placed into three groups, a typical hyrax with bands on the U6’s and 4’s, a bone anchored expander, and a control group. In the bone-anchored group, miniscrew implants were placed between the U6’s and U5’s, 6mm from the midpalatal suture. CBCT was used to measure and evaluate the dental and skeletal effects of expansion. They found that dental expansion was better than the skeletal expansion for both appliances and that there were more dental tipping and vertical changes in the first molar region. The teeth-anchored expander produced extra teeth tipping and dental enlargement on the first premolar region, which was once due to bands being placed on the premolars. Moreover, in 2015, the transverse and dento-skeletal changes of a miniscrew implant-supported maxillary expansion appliance in comparison with other conventional expansion methods were evaluated and compared in growing patients (Chane-Fane & Darqué, 2015; Mosleh et al., 2015; Yılmaz et al., 2015). It has been reported that a bone-borne maxillary expansion could be a suitable alternative to tooth-borne expansion especially when the patients were with vertical growth patterns and reduced anchorage teeth. In addition, tooth-borne maxillary expansion produced dental expansion, buccal rolling, and an increase in nasal width as compared to bone-borne maxillary expansion. They suggested more studies for evaluation of the long-term effects of both approaches with large sample size.

In 2016, Choi and coworkers studied the stability of miniscrew-assisted RME using hyrax expander with 4 miniscrew implants and bands on premolars and molars in young adult patients. They found that nonsurgical miniscrew-assisted RME is a valid treatment option for MTD in young adolescent cases (Choi et al., 2016). While in late adolescent patients, Lin and coworkers reported that the bone-borne expander without surgical assistance can be an effective treatment modality (Lin et al., 2014).

In 2014, Lee and coworkers used finite element analysis to study adult dry skulls for the displacement and stress distribution achieved with the use of bone-borne RME with and without surgical assistance. They found that in comparison to surgical models, non-surgical models exhibited more stress at midpalatal suture. In addition, the amount of stress and displacement along the craniofacial and midpalatal sutures and the teeth was comparable in both surgical and non-surgical models. Therefore, use of minimal surgical intervention for midpalatal suture separation was recommended with the use of bone-borne rapid maxillary expander (Lee et al., 2014). However, in their study, authors evaluated the initial effect of applying a 0.5 mm expansion in the jack screw and did not apply full hyrax activation. In addition, they used a mathematical modeling based on a dry skull that might differ from actual clinical results.

In more recent studies, finite element models were also used to evaluate and compare the stress and strain distribution pattern produced by bone-borne maxillary expander and conventional hyrax RME in the circum-maxillary and midpalatal suture in growing individuals. It has been found that the stresses and strains increased significantly in the entire jaw specifically at midpalatal suture (Figure 2.3) when the bone-borne expander is used, and the expansion achieved was truly skeletal with minimal dental movements in comparison with the conventional hyrax. The bone-borne expander could be more effective considering the objective of suture expansion (Carvalho Trojan et al., 2016; Jain et al., 2017; Mathew et al., 2016). They suggested that expander protocols activation for bone-borne appliances should be carefully studied and adjusted and further experimental studies for suture properties could provide more accurate results.

To date, there have been no studies analyzing the expander protocol activation at once (instant) for bone-borne expansion appliances that might have a significant effect on the amount of sutural expansion and newly formed bone which subsequently affect the amounts of skeletal expansion. Also, no studies have evaluated the effect of sutural corticotomy with bone-borne expansion appliances that is supposed to have positive effects on the amount of skeletal expansion.

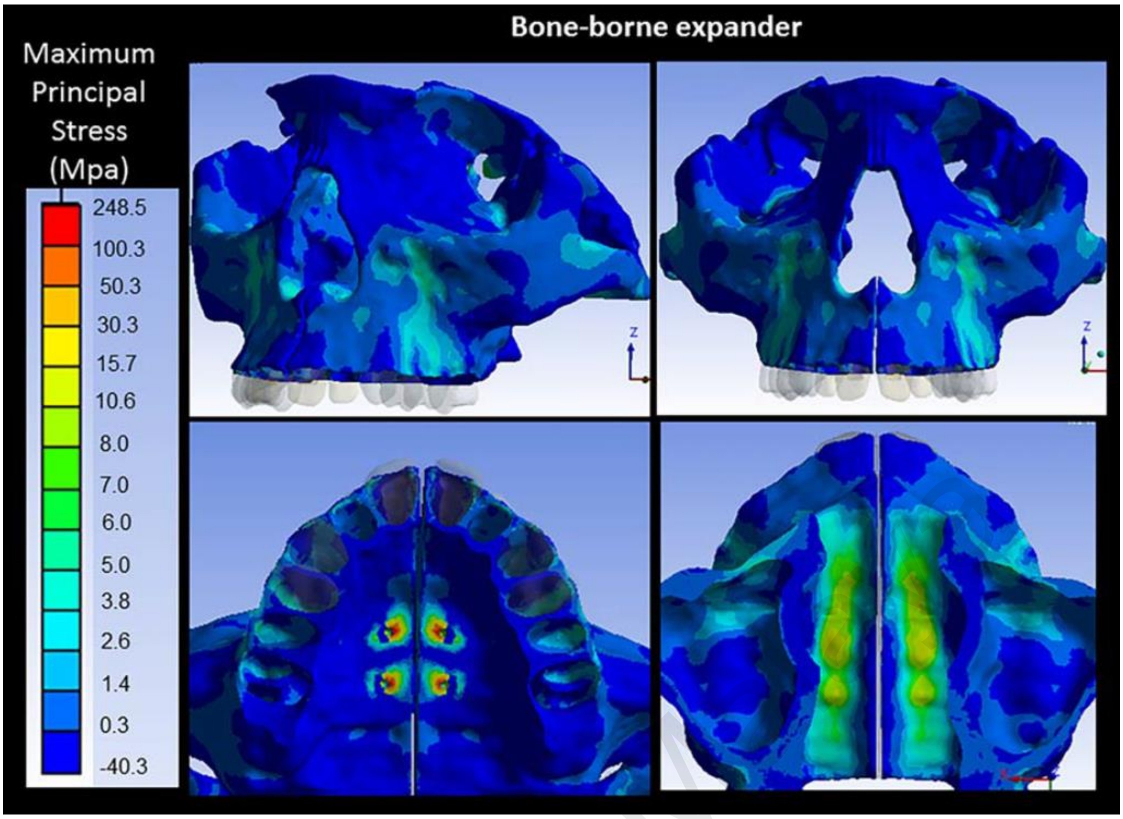


Figure 2.3: The distribution of sutural stresses after force application with bone-borne rapid maxillary expansion to expand mature midpalatal sutures. The higher stresses appeared at midpalatal suture and around implant places (Jain et al., 2017).

2.4.4 Surgical treatment for maxillary transverse deficiency

After reaching the age of skeletal maturity, cases with skeletal discrepancies exceeding 5 mm are not manageable with orthodontic intervention alone. However, orthodontists are able to camouflage cases using orthopedic forces with discrepancies less than 5 mm (Silverstein & Quinn, 1997). Various issues have been associated with use of tooth-borne RME in mature individuals, such as relapse or failure of expansion and periodontal complications associated with these appliances (Suri & Taneja, 2008). Timms & Vero (1981) studied that before establishing the stability 33 to 50% of the maxillary expansion relapses (Timms & Vero, 1981). In adult cases, skeletal resistances are greater which result in excessive forces in teeth leading to root resorption, tipping and other periodontal issues like gingival recession, pain and/or necrosis of mucosal tissues (Bell, 1982; Mommaerts, 1999; Silverstein & Quinn, 1997; Starnbach et al., 1966). These effects occur due to anchorage gained from surrounding teeth. However, problems that are related to the mechanical strain on the teeth can be avoided when the forces are applied directly to the bone. This lead to the development of bone-borne anchorage design in 1999 that can directly apply forces on the bone and consequently avoiding the unwanted dental effects. This anchorage design when used in conjunction with surgical separation of midpalatal suture was documented to achieve maxillary expansion (Harzer et al., 2006; Iida et al., 2008; Mommaerts, 1999; Verstraaten et al., 2010). As a result, the combination of surgical and orthodontic management by using implant anchorages can be used for the widening of the maxilla in skeletally matured patients. However, there is no consensus in the literature regarding the type of distractor (tooth-borne, bone-borne or hybrid type) that should be used for SARME to provide the best dental and skeletal results and stability (Koudstaal et al., 2005; Verstraaten et al., 2010; Vilani et al., 2012). The short-term (post-retention) skeletal and dental changes following bone-borne and tooth-borne SARME has been evaluated and compared using CBCT) imaging (Zandi et al., 2014). They found that the amount and pattern of expansion was not significantly different between tooth-borne and bone-borne SARME. The overall complication rate was negligible in both techniques. Selection of the distraction device for SARME should be based on each individual patient's requirements. Furthermore, in recent study, Kayalar and coworker studied the skeletal, dental, and periodontal effects of tooth-borne and hybrid devices in SARME. It has been found that both Hybrid and tooth-borne devices were effective for SARME with similar V-shaped opening of the suture, and the skeletal results remained stable at the retention period (Kayalar et al., 2016).

There are many advantages of using SARME. It can drastically reduce treatment duration, negligible influences or harms periodontal tissues, can improve nasal airflow, overcome dental camouflage effects, produce esthetic gingival contours while smiling (Swennen et al., 2001), cosmetic enhancement of secondary post-expansion prominence resulting from the buccal hollowing at the site of the lateral wall osteotomy (Bell & Epker, 1976; Starnbach et al., 1966), and avoidance of unnecessary dental extractions (Silverstein & Quinn, 1997).

Orthodontists and surgeons have difference of opinion regarding the indications of SARME. Maxillary expansion is required in a lot of cases, however, an accurate treatment plan for MTD is complicated. This is further complicated due to the different intervention that are described in the literatures about orthodontic and other forms of maxillary expansion. The following were the indications of SARME considering skeletally mature patients with MTD (Koudstaal et al., 2005; Woods et al., 1997):

1. To correct posterior crossbite and increase maxillary arch perimeter when no jaw repositioning is planned.
2. To achieve widening of maxillary arch in preparation of a planned orthognathic surgery.
3. To gain space in non-extraction cases with dentally crowded maxilla.
4. Cleft associated maxillary hypoplasia.
5. Wide buccal corridors.
6. Revision of failed RME by separation of sutures.

2.4.4.1 Surgical technique

Varying techniques focusing two different approaches have been developed. One approach focuses on increased mobility of maxillary bone to cover larger discrepancies but with increased complications. Other approach focuses on a less invasive technique with possible chances of relapse, fractures, and periodontal problems. Zygomaticomaxillary junction is reported to impart primary resistance in the transverse distraction. To achieve separation of zygomaticomaxillary junction a corticotomy from maxillopterygoid junction to the piriform rim and through the zygomatic buttress is done (Figure 2.4A) (Koudstaal et al., 2005). The midpalatal suture is traditionally regarded as the important location of resistance, however, this used to be verified to be untrue by Isaacson and Ingram (Isaacson & Ingram, 1964). Many surgeons perform separation of midpalatal suture to allow mobility and to avoid nasal septum deviation (Figure 2.4B). Several authors describe twin paramedian palatal osteotomies starting from point posterior to incisive canal to posterior nasal spine (Figure 2.4C) (Bierenbroodspot et al., 2002; Booy et al., 2000). Among the site of resistance, pterygoid plates are considered to hold prime importance (Figure 2.4D). However, an increased risk of injuring the pterygoid plexus warrants avoidance of manipulation along these lines. In order to avoid pterygoid separation, v-shaped opening of maxillary halves has been used. Use of V-shaped opening has also been considered as an individual treatment for increased maxillary expansion in anterior and posterior parts. The separation of nasal septum from the palatal base is performed to restrict transferring unilaterally part which leads to the alterations in nasal airflow (Figure 2.4E) (Koudstaal et al., 2005).

Freitas and colleagues concluded that osteotomies in zygomatic pillars in combination with midpalatal osteotomy was a suitable treatment for MTD (Freitas et al., 2008). Northway and Meade described that buccal corticotomy combined with midpalatal osteotomy resulted in increased palatal width (Northway & Meade Jr, 1997). Shetty and colleagues demonstrated that pterygomaxillary osteotomies combined with midpalatal osteotomy is required to successfully predict MTD treatment, they also found that bilateral zygomatic buttress osteotomy alone appears inadequate (Shetty et al., 1994). In light of the literature available regarding use of different surgical procedures and combinations, debates in the favor and against maximal mobility run parallel. However, prime importance has always been given to minimally invasive surgical procedures (Koudstaal et al., 2005).

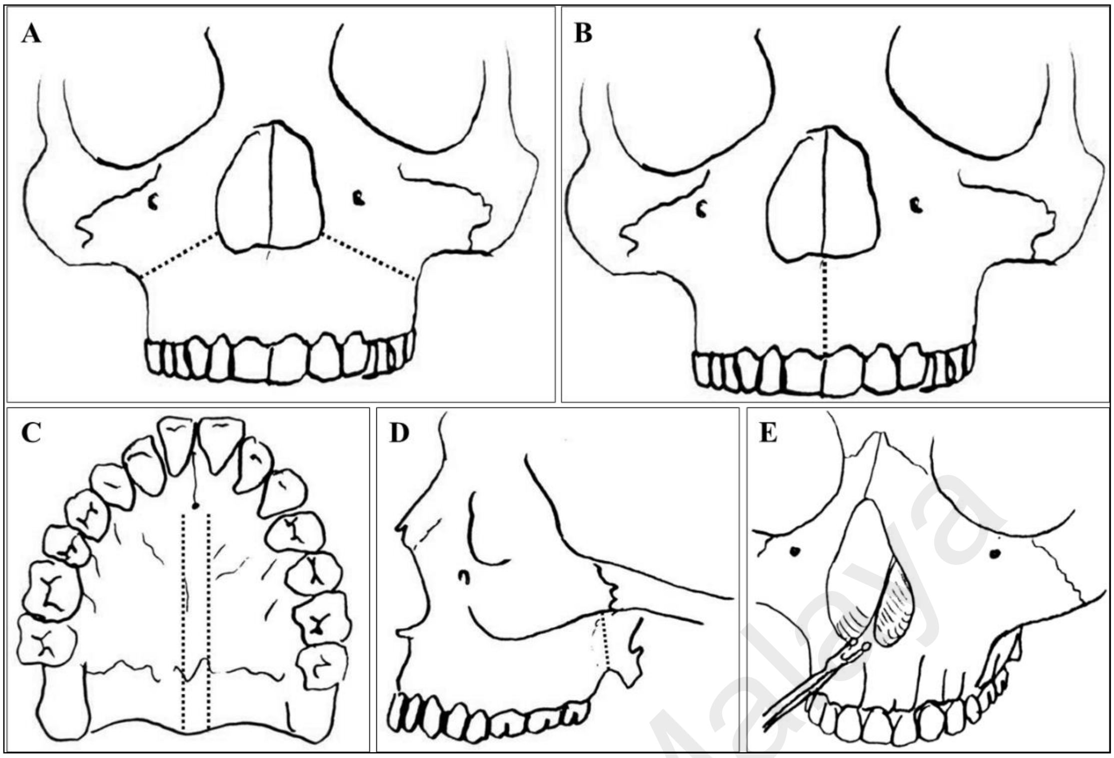


Figure 2.4: Schematic drawing of various surgical techniques showing (A) the corticotomy from the piriform rim to the maxillopterygoid junction, (B) the osteotomy of the midpalatal suture, (C) the two paramedian palatal osteotomies from the posterior nasal spine to a point just posteriorly of the incisive canal, (D) the osteotomy of the pterygoid plates, and (E) the release of the nasal septum with the use of a septum osteotome.

2.4.5 Piezoelectric surgery for treatment of maxillary transverse deficiency

Since 1988, piezoelectric ultrasonic devices had been used for biomedical purposes. General applications of piezoelectric devices include periodontal surgeries, impaction removals, apical surgeries, minor oral surgeries (Vercellotti, 2004; Vercellotti et al., 2001b), and bone expansion (Metzger et al., 2006; Schlee et al., 2006). Piezocision is a different and modern modality for treatment planning in various medical fields. Its application has been developed gradually over time. Initially, the piezocision was introduced as minimally invasive surgical modality, since then it has been used for corticotomies and currently, scientists are finding new applications in orthodontics and multi-disciplinary care. Orthodontists have focused on achieving selective anchorage control over teeth to establish new treatment modalities to broaden the scope of conventional orthodontics. Piezocision can be applied locally, generally, and or sequentially. Use of piezocision for extensive corticotomies might provide a suitable alternative to SARME (Dibart et al., 2015).

2.4.5.1 Technical characteristics

Piezoelectric osteotome is attached to handpiece which operates through a transducer that converts electricity to ultrasonic waves, producing ultrasonic vibrations which perform selective cutting upon contact. Following modulation through the transducer ultrasonic waves pass from the inserts which then oscillate in a linear pattern. With a power ranging from 5-16W and frequency ranging from 25 to 30 kHz the oscillation amplitude ranges between 60 and 200 mm. The ability to distinguish between hardness of different tissues depending upon their mineralization is a characteristic feature of piezo-surgical instrument. This, in turn, averts potential contact injury to oral tissues. This type to instrumentation ensures 'bloodless' field of surgery and it is also equipped with a high-flow saline irrigation attached to the instrument to allow instant cool-down of bone while undergoing osteotomy (Vercellotti, 2004).

2.4.5.2 Advantages of piezoelectric bone surgery

Contemporary piezoelectric bone cutting technique (piezosurgery) has several advantages. The slicing end-piece can move horizontally or vertically to attain high precision, clean cuts with no wrinkled edges. Potential chances of heat-induced trauma and bacterial contamination from the piezoelectric devices are reduced by attachment of high-flow saline irrigation (Bacci et al., 2011; Berengo et al., 2006). The characteristic difference of using piezoelectric device over conventional cutting instruments is its ability to cut different types of tissues selectively without damaging other types of tissues by setting different frequencies for specific tissues. Operating frequencies above 50 kHz can cause damage to delicate tissues like nerves surrounded by bone and damage to hard tissue can occur at 25-29 kHz (Vercellotti, 2000; Von See et al., 2012). The piezo surgery device is beneficial for the minimal invasive interventions in cranial and maxillofacial surgery with minimization of surgical trauma (Bonitz et al., 2009). It ensures clean operation area, better visibility of the operated tissues and low tissue necrosis due to the cooling system with the physiological saline solution (Vercellotti et al., 2001a).

2.4.5.3 Osseous response to piezoelectric surgery

Piezo surgery attains a better postoperative bone healing process because of the elevated amount of Transforming Growth Factor-Beta 2 (TGF-β2) and Bone Morphogenetic Proteins 4 (BMP-4). In addition, it ensures higher control of the inflammatory process through releasing specific cytokines (Labanca et al., 2008). Histologic slides have displayed live osteocytes and absence of necrosis due to coagulation while examining the cutting surfaces of bones (Figure 2.5) (Vercellotti et al., 2001a). In comparison with the conventional rotary handpiece, piezo instruments have comparable results in terms of patient temperature perception and vitality of the pulp can also be protected with its use. Postoperative consequences like swelling and hematomas can be avoided with its use (Robiony et al., 2007).

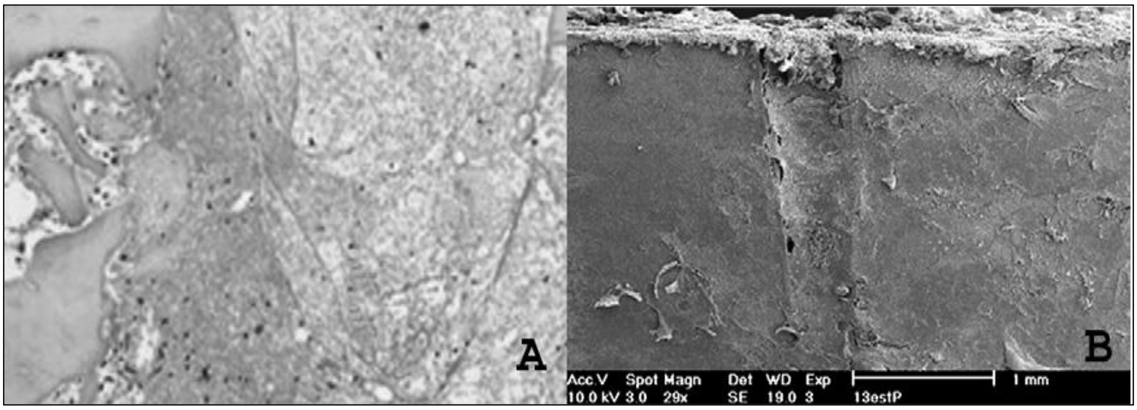


Figure 2.5: Microscopic view of a bony surface after piezoelectric cutting: photomicrograph (hematoxylin and eosin stain, original magnification x10) (A) and electron micrograph (B), showing absence of necrosis and presence of live cells (Vercellotti et al., 2001a).

2.4.5.4 Piezoelectric sutural corticotomy

For achieving a symmetric expansion of maxilla, midpalatal suture is considered an important area of resistance, however, pterygoid junction, zygomaticomaxillary buttress, and piriform aperture are also considered area of resistance (Aziz & Tanchyk, 2008). The split corticotomy of midpalatal suture for maxillary expansion was first described by Brown, and SARME is considered the treatment of choice for MTD cases in skeletally mature adults (Brown, 1938). In 2014, Lee and colleagues conducted a study on dry skulls of adult human to evaluate five different designs of RME: type A consisting of a tooth-borne conventional hyrax expander, type B consisting of a bone-borne expander, type C consisting of bone-borne expander in combination with midpalatal suture, type D consisting of bone-borne expander in combination with pterygomaxillary sutures split, and type E consisting of bone-borne expander in combination with LeFort I corticotomy. They concluded that type A and B showed prominent expansion in anterior region with diminished results in the posterior region. They found that higher stresses were detected in type A and B modules due to avoidance of surgeries. Type C, D, and E showed similar stress and displacement patterns along teeth and different sutures. Therefore, it was suggested that bone-borne RME with midpalatal suture split might yield superior results with minimal surgical intervention. A further study might be required to evaluate the total treatment effect when applying full activation of the jack screw (Lee et al., 2014). Furthermore, Sant and coworkers in 2016, concluded in their study that for achieving increased effectiveness midpalatal suture split should be included in the surgical technique. In his study, the cases treated with the surgical technique avoiding midpalatal split was not successful and patients presented with discomfort and pain during activation of expander (Sant'Ana et al., 2016).

Conventionally, the midpalatal splitting is achieved by using rotating and drilling instruments, such as the burs, osteotome, or chisel. The rotating instruments are potentially injurious, due to the production of excessively high temperatures during osseous drilling, which can produce marginal osteonecrosis and impair bony regeneration (Kerawala et al., 1999). Furthermore, when using drilling instruments, there is a higher risk for damage to the surrounding and enveloped tissues in more complicated anatomic locations (Rupprecht et al., 2003). Moreover, the vibrations produced by handpiece and rotary instruments can be disturbing for the patient (Hohlweg-Majert et al., 2010). A careful surgical technique is necessary to avoid such complications and high-pressure saline irrigation is used to avoid frictional heating generated by drilling with handpiece (Robiony et al., 2004). One of the minimally invasive surgical techniques is piezoelectric surgery, which was mentioned in the previous sections of this review.

Several clinical applications of piezoelectric surgery in SARME and Le Fort I osteotomy are summarized in appendix B. Landes and coworkers performed a large study on patients in which orthognathic surgery was performed by piezosurgery. They reported that in cases of Le Fort I osteotomy, additional use of chisels was necessary during final separation of the pterygomaxillary suture in 33% of cases, and during separation of the nasal septum and dorsal lateral nasal cavity walls in all cases because the piezoelectric tools were unable to reach the desired position. Piezoelectric osteotomy reduced blood loss and inferior alveolar nerve injury with no extra time investment (Landes et al., 2008a; Landes et al., 2008b). Munoz-Guerra and colleagues performed a combination of Le Fort I osteotomy with bilateral sinus lift with inlay bone augmentation in a severely atrophic maxilla using piezosurgery. They concluded that piezosurgery assisted in accurate placement of implants and they were successful in achieving the amount of bone augmentation as required (Muñoz-Guerra et al., 2009). While performing SARME, a split is created between central incisor by using thin, tapered, and angulated tip. The pterygomaxillary junction is separated using piezosurgical instrument with minimal risk of damaging the descending palatine artery. The pterygomaxillary separation is considered important to accomplish a SARME and/or Le Fort I osteotomy, to achieve posterior expansion. Piezosurgical instrument can cut precisely between teeth, sparing maximum amount of adjacent bone and preserve vitality of teeth in the region effortlessly (Robiony et al., 2007). Furthermore, Rana and coworkers performed a randomized prospective trial on 30 patients (18-45 years old) in which the SARME was achieved by using piezoelectric surgery and oscillating saw. Le-Fort I without the down-fracture technique was standardized as the surgical procedure. They found that use of piezoelectric device to cut bone and protect adjacent tissues was possible. They concluded that piezoelectric device is sooner or later going to modify many conventional operating procedures (Rana et al., 2013). Robiony and colleagues performed endoscopically assisted piezo-osteotomy in 13 patients aged 15-26 years. They performed two osteotomies, the first one from the zygomatic buttress until the pyriform rim including 1 cm osteotomy of lateral nasal wall and the second one from the zygomatic buttress to the pterygomaxillary junction. They concluded that the procedure described is minimally invasive and has advantages over the usual technique in providing direct vision, the creation of a precise and safe osteotomy, minimal bleeding risk, and decreased dissection (Robiony et al., 2014).

In 2015, Koszowski and colleagues published a case report on maxillary stenosis in an adult patient treated with SARME using piezosurgery. The surgical technique included maxillary cortical osteotomy from the distal surface of maxillary tuberosity to the lateral piriform aperture on the right side above tooth apices. They reported that the osteotomy in the maxillary lateral segment using piezosurgery enabled micro-invasive operation with minimal risk of damage to the maxillary sinusal mucosa (Koszowski et al., 2015). From this review, we found that piezosurgery was successfully used in various osteotomy techniques in the SARME. However, no literary evidence regarding the outcome of piezoelectric sutural corticotomies on SARME has been found till date.

2.5 Stability of surgical treatment compared to non-surgical treatment

In pre pubertal children and teenagers, non-surgical RME usually relapses about one-third of the total expansion achieved across the first molars (Lagravere et al., 2006). Posterio-anterior cephalometric radiographs revealed that 50% of the expansion achieved via RME was skeletal and the other half of the children had dentoalveolar (Lagravere et al., 2005).

Handelman and colleagues (2000) concluded in their study that 56% of the cases from young age exhibited skeletal expansion as compared adult age group showing expansion in only 18% of cases (Handelman et al., 2000). Baccetti and colleagues found that cases treated before reaching skeletal maturity showed expansion up to 3 mm, however, cases treated after skeletal maturity showed expansion up to 0.9 mm (Baccetti et al., 2001). From previous studies, we can estimate that the nature of expansion shifts from skeletal to dental in skeletally mature individual, which can cause detrimental effects on the periodontal tissues surrounding the anchored teeth.

In 2008, Chamberland and coworkers determined that a mean 3.47 mm of skeletal expansion relapsed, which is 68% of the mean dental enlargement (5.12 mm). The relapse of dental expansion is comparable in SARME and non-surgical RME, however, the skeletal expansion in SARME is stable in comparison to non-surgical cases (Chamberland & Proffit, 2008).

2.6 Orthodontic miniscrew implant

The miniscrew, which was originally designed to fix bony segments, has shown great promise as a simpler and more versatile solution for obtaining absolute anchorage. Many authors have reported successful use of miniscrews in a wide range of orthodontic tooth movements (Costa et al., 1997; Kyung et al., 2003). Miniscrews are used as temporary fixtures in bone and their greatest advantage lies in their small size, which permits rapid and atraumatic placement in almost all sites within the mouth. In the past decade, there have been rapid advances in the development of miniscrews and they are increasingly used in orthodontics.

2.6.1 Research and development of miniscrew implant

The development of miniscrew implant in orthodontics are summarized in the Table 2.2. In 1945, Gainsforth and Higley first introduced the concept of skeletal anchorage using vitallium ramal screws in dogs (Gainsforth & Higley, 1945). This attempt failed, as did almost all implants of the era, because there was inflammation around the vitallium screw, leading to loosening and loss of the implants.

In 1969, Brånemark and colleagues introduced the concept of osseointegration in dentistry, using pure titanium implants (Brånemark et al., 1969). Brånemark defined osseointegration as “living bone in direct contact with a loaded implant surface” (Branemark, 1977). This definition is based on observations made at the light microscopic level. However, few clinicians envisaged the use of titanium implants in orthodontics at that time. It was not until the 1980s, that several animal studies on the use of titanium implants in orthodontics reported successful results (Roberts et al., 1989; Roberts et al., 1984). These animal studies were followed by a case report in which an osseointegrated titanium implant in the retromolar region was used as anchorage to move two molars 10–12 mm mesially through a post-extraction atrophic alveolar ridge (Roberts, 1994). Further research by Turley and colleagues suggested the possibility of using the endosseous implant as an anchor in palatal expansion in monkeys (Turley et al., 1980), in which they expanded the palate by applying 425 g of force on bioglass-coated ceramic implants. In 1983, Creekmore reported a case in which a vitallium implant was utilized for anchorage by placement underneath the anterior nasal spine. A light elastic thread was tied from the head of the screw to the arch wire after 10 days of placement of the implant to intrude the maxillary incisors (Creekmore, 1983). This early loading of an implant, without the usual wait for osseointegration, was to become a major feature of the later use of miniscrews.

A next step in adapting implant technology in orthodontics was the development of short conventional implants to be placed in the midline of the palate (midpalatal implants). These are now a well-recognized and documented source of anchorage, but are still relatively expensive and complex. They need careful placement in the palatal vault to ensure sufficient bone depth and no contact with the roots of adjacent teeth and are therefore relatively inconveniently situated for a palatal arch to take advantage of them. These implants are usually 6 to 10 mm in length and 3 to 4 mm in diameter. Traditionally, 10 to 12 weeks of stabilization period is required before applying force to the implants

(Celenza & Hochman, 2000; Wehrbein et al., 1996). Tinsley and colleagues gave an excellent description of the typical use of these implants (Tinsley et al., 2004). Other practical tips can be found in two articles by Cousley and Parberry (Cousley & Parberry, 2005; Cousley, 2005). Wehrbein and coworkers reported a case in which absolute anchorage was provided by a palatal implant with a length of 4 to 6 mm and diameter of 3.3 mm, which required far less extensive surgery (Wehrbein et al., 1999).

In 1995, Block and Hoffman introduced another type of skeletal orthodontic anchorage called onplants. They used a 10 mm wide and 2 mm thick hydroxyapatite coated titanium disk. It was inserted through a subperiosteal tunnel prepared through a paramarginal incision, which is rather extensive soft tissue surgery (Block & Hoffman, 1995). Onlplants are osseointegrated to the bony surface and designed to be left unloaded for 4 months. The need for osseointegrated implants of any type in the palate has been greatly diminished by the development of miniscrews. Because of the anatomic shape of the nasal crest, which extends between the anterior and posterior nasal spines, the midpalatal area is now considered to have adequate bone for retention of the miniscrew implant throughout its length. This overcomes the need for either an onplant or a short conventional osseointegrated implant which is restricted to just one palatal site in the anterior of the palate (Fee Jr, 1991; Kyung et al., 2003).

The late 1990s saw the introduction of miniscrews as temporary anchorage devices. In 1997, Kanomi reported using a mini-implant for orthodontic anchorage. He used a mini bone screw with 6 mm length and 1.2 mm diameter, which was designed for fixation of bone plates in plastic surgery. He drilled the bone before placing the miniscrew implant and waited 4 months for osseointegration before loading the implant. Opinion has since varied on the optimum timing of initial loading. He prefers to load an orthodontic miniscrew 1 week after the surgery when the soft tissue has healed (Kanomi, 1997).

Table 2.2: History of development of the orthodontic miniscrew implant.

Year of introducing	Authors and reference	Type or name of screw	Notes
1945	Gainsforth and Higley (Gainsforth & Higley, 1945)	Vitallium ramal screws	The attempt was failed due to the inflammation around the vitallium screw, leading to loosening and loss.
1969	Brånemark and colleagues (Brånemark et al., 1969)	Osseointegrated pure titanium screw (endosseous implant)	In 1980, after several animal studies on the use of osseointegrated titanium implants in orthodontics reported successful results.
1995	Block and Hoffman (Block & Hoffman, 1995)	Onplant (disk-type onplant)	Restricted to just one palatal site in the anterior of the palate.
1996	Wehrbein, et al. (Wehrbein et al., 1996)	Short conventional osseointegrated implant	Needs careful placement in the palatal vault to ensure sufficient bone depth and no contact with the roots of adjacent teeth. Restricted to just one palatal site in the anterior of the palate.
1997	Kanomi (Kanomi, 1997)	Miniscrew implant	Miniscrews have become established as practical, inexpensive, highly versatile sources of orthodontic anchorage.

2.6.2 Placement of miniscrew implant in the midpalatal region

An excellent site for the miniscrew implant placement with regards to hard and soft tissue is the midpalatal bone area. Highly dense cortical plate and the thin layer of keratinized soft tissue is an advantageous site for retention of implant (Lee et al., 2004). 5 mm is the shortest thread length of miniscrew considered adequate. Although the nasal crest is present on its dorsal aspect, the bone thickness is limited and cannot be accurately measured on a conventional radiograph. A miniscrew with greater length could penetrate into the nasal cavity. Since the midpalatal region is composed of hard, dense cortical bone, a miniscrew does not have to be embedded too deeply in the bone for adequate stability.

When selecting the connecting bur, the depth of the palatal vault and the angle of placement need to be considered. A deep palatal vault requires a longer connecting bur (24 mm) to avoid collision of the handpiece with the upper incisors during placement. Regarding the direction of placement, the miniscrew should be inserted perpendicular to the roof of the oral cavity. However, in deep palates, the miniscrew may have to be inserted slightly from posterior to anterior direction in the sagittal plane (Figure 2.6) and the length of the miniscrew engaged in the bone is greater. This not only improves its retention by increasing the contact between the screw and the bone but also reduces the risk of perforation of the nasal cavity. It is also often easier to engage an elastic module on a miniscrew inserted in this way. A short hand driver or torque driver may also be used to place a miniscrew in the midpalatal region, but it can be difficult to turn the handle against the highly dense midpalatal cortical bone. Quite often, the force generated manually may not be enough to initiate insertion. Also, if the handpiece is used alone, a transpalatal arch may be in the way. The path may be deflected and cause breakage of the miniscrew.

Usually, a motor-driven handpiece and short hand driver are used in combination; the handpiece is used in the initial stage of insertion when a high torque, or strong rotating force, is required. After more than half of the threaded part has been inserted into the bone, the short hand driver is used to drive in the rest of the miniscrew. The advantage of using the hand driver is the ability to have tactile sense during insertion. The subtle bone resistance can be detected and miniscrew breakage due to too heavy a rotating force is prevented (Paik, 2009).

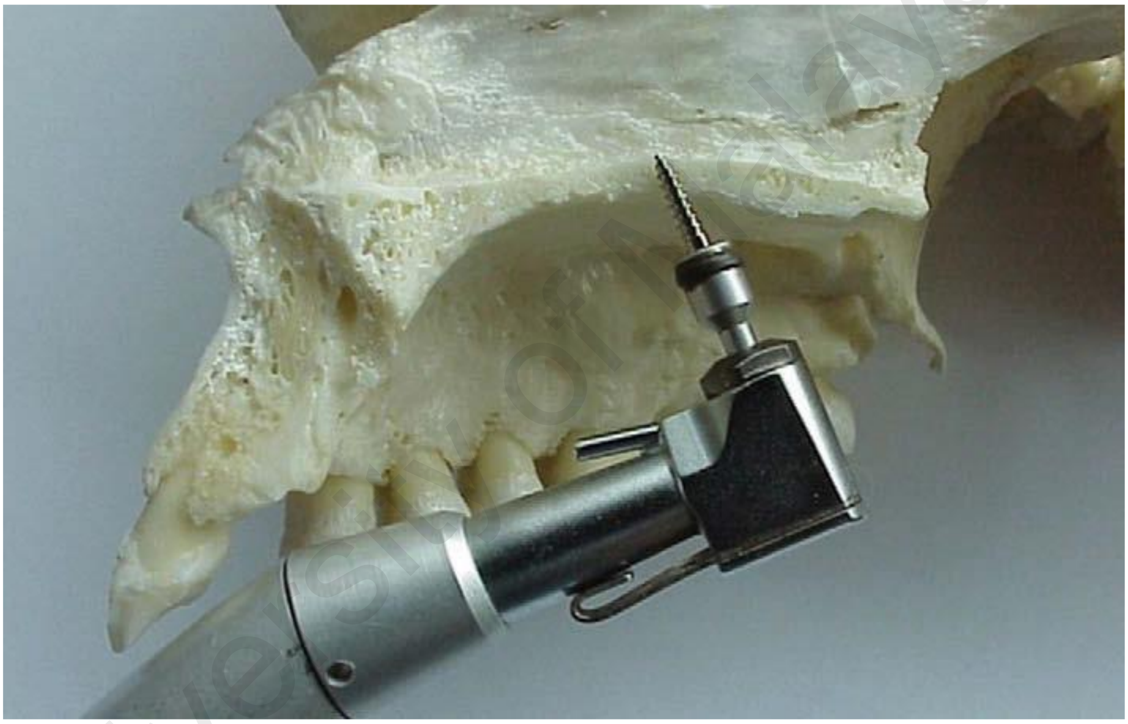


Figure 2.6: Direction of insertion of the miniscrew in a deep palate (sagittal view) (Paik, 2009).

2.7 Cranial sutures

Calvarial bones are interconnected with connective tissue interfaces called cranial sutures, which primarily allow expansive growth of interconnected bones and brains. Sutures act as joints and transmit imparted mechanical loads originating from natural activities like mastication or exogenous sources like orthopedic forces (Mao, 2002; Mao & Nah, 2004). Sutures consist of two bone fronts which are composed of osteoblast cells which sandwich highly vascular matrix containing mesenchymal and fibroblast-like cells (Opperman, 2000). Sutural osteogenesis leads to the initiation of a physiological phenomenon which contributes to the maintenance of suture vitality and mesenchyme which in turn prevents complete mineralization of the suture (Warren & Longaker, 2001). Suture development at different levels of organization is greatly influenced by the exogenous mechanical stresses (Mao, 2002).

Longitudinal development of major components of skull is facilitated by the sutures, without which the skull bones cannot elongate however they might grow thicker. Sutural biology has gained interest of researchers and scientists focusing the developmental studies ranging from paleontological form to genetic patterning. Clinicians have focused to impart mechanical forces by using orthopedic appliances to modulate sutural development for treatment of myriad varieties of skeletal deformities (Mao, 2002). Skull expansion has been thought to result from the tensile stresses generated from brain expansion causing the separation of cranium subsequently allowing sutural development, however, studies focusing anabolic sutural responses generated from mastication and/or exogenous stimuli were identified to be of compressive nature which were found competent of modulating sutural growth (Mao et al., 2003).

2.7.1 Mechanical properties of sutures

The change in length of a material object divided by the original length that specific material object is defined as strain, which is usually described in unit of percentage. When a load is applied on an object, it experiences a certain amount of stress and strain. The strain is measurable in contrast to stress and is sensed by cells. For elastic objects, the division of stress by strain is a constant termed as elastic modulus which is considered approximately equivalent to stiffness (Carter & Beaupré, 2007).

Sutures act as movable joints in certain nonmammalian vertebrate which influence movement ranges rather than force transmission. In contrast, sutures behave differently in mammals, by transmitting loads of forceful mastication. Their construction features interdigitation and/or overlapping at the bony fronts (Gussekloo et al., 2001). Skeletal mature sutures have characteristic fibrous interface that acts as a union which is capable of absorbing compressive and tensional forces. These sutures can rival the strength of adjoining bones (Herring & Teng, 2000). Sutures absorb most of exogenous forces and are highly flexible, therefore resist most of the deformation loads from different directions at different rates as compared to rigid bones (Herring, 2000).

Within the two mesenchymal layers, the sutures contain proteoglycans, water and collagenous fibers in an extracellular matrix providing the viscoelasticity to the sutures. Viscoelasticity is the ability of the sutures to absorb exogenous energy. The mechanical properties of sutures are greatly dependent on the duration, rate, and linearity of load applied as it dynamically changes the arrangement of extracellular matrix. A possible explanation regarding the linearity might be that the arrangement of fibers is able to resist a particular type of load either compression or tension and not both at the same instance (Herring, 2008). Different suture depending on their morphologies and fibrous array might exhibit different mechanical properties, like densely interdigitated sutures, might have a higher elastic modulus.

The age related changes influencing the mechanical properties have been extensively studied. During embryonic and immediately post-natal stages of development, sutural ligament is under-developed which later act as the primary force bearing components (Herring, 2008). Later stages of sutural development are marked by the characteristic bony interdigitation. It can be assumed that the post-natal sutures are therefore less rigid and feebly capable of absorbing exogenous energy (Henderson et al., 2005). The mechanical properties of different sutures differ depending upon their location and age maturity. In a rat study, the sagittal or coronal sutures were stiffer in comparison to the posterior interfrontal suture (McLaughlin et al., 2000). Interestingly, a study described that the only suture which underwent fusion in rats was the posterior interfrontal suture (Opperman, 2000).

The mechanical properties of bone-suture complex are dependent on the arrangement of fibers. A study of miniature pigs was conducted in which isotropic suture models were tested under tension and compression which showed that the sutures had the capability to optimize fibrous arrangement to experience different types of load applied. Orthotropic models had high strain energy in comparison to isotropic models and isotropic models had a compression-resistant fibrous arrangement. The orthotropic and isotropic models represent the two different morphologies of sutures that depicted a central network of collagen fibers with the sutural margin fibers directionally arranged. It is thought that an intermediate arrangement could benefit both extremes of the suture models. Suture strain energy showed a decrease with the increase in sutural interdigitation in the isotropic models (Jasinoski et al., 2010). However, the rate of load applied (Margulies & Thibault, 2000) and the bony interdigitation (Jaslow, 1990) can also affect the amount of energy absorption, which require further investigation. An increase in interdigitation index allows increased transfer of load which is noted from a shift from cranial sutures to long bone sutures.

2.7.2 The response of sutures to stress application

With the application of load to a solid object, molecular momentum of the specific object is altered. This momentum is translated into three possible variants: change in velocity of the object, deformity of the object, or both combined. Forces cause deformation in the individual cells resulting a change in the entire connective tissues.

Different types of forces, tissue architectures and materials result in complex deformation patterns (McLaughlin et al., 2000).

Having established that intrinsic biomechanical differences exist among the cranial sutures. In theory, this difference can result from fibrillar structural differences, fibrillar orientation differences, or both. The Hurschler model shows the effect of differences in orientation. The mathematical modeling of changes in tensile behavioral secondary to both fiber heterogeneity and variable fiber orientation is more difficult (McLaughlin et al., 2000).

2.7.2.1 Sutural stress response in the form of morphological changes and interdigitation

Impacts and shocks to craniofacial complex are absorbed by sutures. The amount of energy absorption by a suture can be correlated its level of interdigitation (Jaslow, 1990). Interdigitation increases the surface area of the bony interface for collagen fibers to align along the bony fronts and in turn alleviate the stresses (Herring, 2008; Jaslow, 1990). Sutures that resist tension have simple morphology and less interdigitation at the interface as compared to the sutures that resist compression (Herring & Mucci, 1991).

Sutural width increases laterally when under tensile stresses, the magnitude of the applied stress is directly proportional to the lateral growth (Yen et al., 1989). Vardimon and colleagues found that the radiolucent area of sutures was increased 12-folds under orthopedic forces, while at the retention stage, sutures width was reduced 9-folds than the original width, and ossification was increased significantly. They found that bone formation increased under tensile stresses and prevented mineralization of bony fronts, while after cessation of tensile forces the sutural ossification commenced (Vardimon et al., 1998). Tensile stresses applied at palatal sutures resulted in farther apart displacement of the lateral cartilaginous layers and a decrease in width as bone replaced the cartilage (Kobayashi et al., 1999). The presence of resting, reversal and cement lines confirmed the bone remodeling activity at the sutures (Movassaghi et al., 1995; Nanda & Hickory, 1984). Another study reported that tensile stresses and irradiation might alter sutural morphology (Sasaki et al., 2003). In their study, they created two holes lateral to the sutures and found that the distance increased under tensile stresses. They also noticed increase in vascularity around the suture and elongation of the transverse fibers in vicinity of the suture. They found that the sutures responded to the exogenous forces exerted on them, however, the source of response, whether, inflammatory response, expansive load, irradiation, or a combination of these factors remained unclear, as the control group was kept unexposed to any of the forces. Knockout mice which had myostatin-deficiency had a higher muscle mass compared to wild-type mice. Knockout mice showed increased osteoblastic activity which can be attributed to the greater masticatory force due to increased muscle mass. Interestingly, knockout mice had higher interdigitation in sutures, however, during mechanical separation these sutures had lower stiffness than wild-type mice. A possible explanation could be the accelerated formation of connective tissue in the interdigitations, which exhibited more flexibility. The knockout mice suture did not show higher breaking strength which can be attributed to the lower stiffness (Byron et al., 2004).

Compressive stresses have been reported to cause structural and morphological alterations in the suture. The sutural width increases significantly and more Howship's lacunae are found on the sutural bony fronts. Bone deposition and blood vascularization increases around the sutures. These changes are permanent, fibrous organization around sutures takes places and no significant remodeling of the bone occurs for up to 2 months. Experimental studies on the sutures revealed that the sutures were significantly wider and complex as compared to the controls (Droschl, 1975). Sutures under compressive forces had abutting morphology and stunted growth eventually became obliterated. Obliquely oriented fibers were found to resist different modes of strain applied. It was found that high compressive strain led to increase in sutural interdigitation, with absence of bone resorption and sutural interdigitation remained unchanged under tensile stress (Rafferty & Herring, 1999).

2.7.2.2 Proliferative changes and sutural cell differentiation in response to stress

Cellular activity and count has been closely related with the changes in sutural width which occurs in response to stress. Different types of stress act differently upon sutures causing narrowing or widening, however, it has been noticed that widening of sutures surprisingly resulted in proliferation of osteoclastic cell lines (Hickory & Nanda, 1987). Difference of ratio between osteoclasts and osteoblasts determines the status of a suture whether it is productive or reproductive phase. Mechanically stressed sutures showed changes in the cellular metabolic activity (Hickory & Nanda, 1987; Meikle et al., 1984). Collagen fiber synthesis has been also correlated with the amount of tensile load applied on the cranial suture (Hickory & Nanda, 1987). It was found that even at low magnitudes of tensile stresses, increase in the vascularity, cellular activity and proliferation was noticeable (Hirukawa et al., 2005; Kopher & Mao, 2003; Movassaghi et al., 1995; Sasaki et al., 2003). The fibroblast cell count has also been found sensitive to the magnitude of force applied as it increases rapidly in response to an increased magnitude. Any mechanical forces applied to the surface of vault are transferred to the bony fronts of sutures as tensile stresses, the bony fronts are composed of fibrous tissues that have considerably higher elasticity than the surrounding bones (Herring & Ochareon, 2005). If a metal spring which has an ectrocranial insertion applies force, the response to force is pronounced on the ectocranial surface in comparison to the endocranial surface. A possible explanation for the increased response from ectocranial is that when a force is applied to any curved plane, increased magnitude of force is experienced on the outer surface of the curve (Steenvoorden et al., 1990; Sun et al., 2004).

A fact to be noted is that in the absence of any exogenous stresses on cranial sutures, these sutures still absorb significant amounts of tensile stress. These stresses are due to the growth of brain and the functional activity of muscles like mastication (Herring, 2000). The brain growth imparts stresses on the suture by separation forces resulting expansion (Herring & Ochareon, 2005), and by increasing pressure in the endocranium resulting in the bone remodeling (Byron et al., 2004). Increase in pressure results in compressive stresses at the endocranium causing bone resorption and tensile stresses at the ectocranial resulting in bone deposition. The process of bone remodeling despite of being slow impart stresses on the bony fronts causing the sutural growth response.

Bone deposition also occurs in response to the functional activity of craniofacial muscles (Herring, 2000). It was found that osteoprogenitor cells increased in number in an instant response to high magnitude of tensile stress and then decreased over time (Southard & Forbes, 1988). In contrast, the compressive stress applied to sutures caused an increase in the number of osteoclast and leading to an eventual decrease in sutural width (Droschl, 1975).

Tensile forces applied on the midpalatal suture can alter the differentiation pathway of osteo–chondro-progenitor cells. The width of the lateral cartilaginous layer decreases and is replaced by bone, however the width of the precartilaginous layer increases and displaced laterally due to the fibrous arrangement at the center of the suture (Takahashi et al., 1996). Tensile stresses induce osteoblast differentiation subsequently causing bone formation (Ikegame et al., 2001). Compressive stresses also induce cell differentiation in the pre-cartilaginous and cartilaginous cell layers and increased expression of collagen type I, II, and X (Saitoh et al., 2000).

The impact of shear load on cell multiplication has not been generally contemplated. This is because of the trouble of deciding the correct amount and areas of the shear loads at a suture. It has been accounted for that shear load was detected on concave surfaces during the use of tensile loads on sutures (Zhang et al., 2002). This perception may add to the clarification of the resorption found on concave surfaces where osteoclasts focus and confine to play out their action (Byron, 2006).

2.8 Bone remodeling

Bone is a well-organized, living tissue that is always adjusting to metabolism and necessary mechanical demands. Since it is a mineralized tissue, all adjustments happen along vascularized periosteal surfaces using uncoupled anabolic and catabolic modeling. Modeling changes the size, position, and shape of bones responding to mechanical loading and/or wounds. Bone remodeling is the turnover of bone that is identified with bone development, skeletal upregulation, and metabolism of bone minerals. There are important differences between bone remodeling and modeling that are pertinent to clinical practice. In the orthodontic field, the expression "bone remodeling" is usually connected to every bone change. This is an awful semantics issue that blocks a fitting depiction of the bone physiology and biomechanics related to clinical treatment. Bone modeling is the mechanically interceded methods for skeletal adjustment that is the physiological grounds of orthodontics and dentofacial orthopedics (Roberts et al., 2004). Moreover, bone remodeling happens in two stages: resorption of the current mineralized bone network by osteoclasts took after by development of new bone by osteoblasts. The procedure occurs at spatially discrete foci, and the cell groups included is known as the basic multi cellular unit (BMU) as appeared in Figure 2.7. The active BMU quantity and the relative measures of bone resorbed and shaped inside individual BMUs decide the rate of bone turnover (Bringhurst et al., 2005; Seibel et al., 2006). In cortical bone, the

BMUs burrow through the tissue, though in cancellous bone, they move over the trabecular surface. The procedure of bone remodeling is started by constriction of the lining cells and the enrollment of osteoclast. These precursors develop into multi nucleated, dynamic osteoclasts that intercede bone resorption. Osteoclasts embed to bone and hence metabolize it by proteolytic processing and fermentation. As the BMU propels, osteoclasts leave the resorption site, and osteoblasts move in to cover the emptied territory and start the procedure of new bone arrangement by discharging osteoid, which in the long run is mineralized into new bone. After osteoid mineralization, osteoblasts smooth and shape a layer of lining cells over new bone. The remodeling of bone happens along the lines of forces created by mechanical loads. The signs from these mechanical loads are detected by osteocytes, which transmit signs to osteoclasts and osteoblasts or their precursors. One such sign made by osteocytes is sclerostin, an inhibitor of wingless-sort (wnt) signaling. Mechanical loads stunt sclerostin generation and consequently increase bone arrangement by osteoblasts.

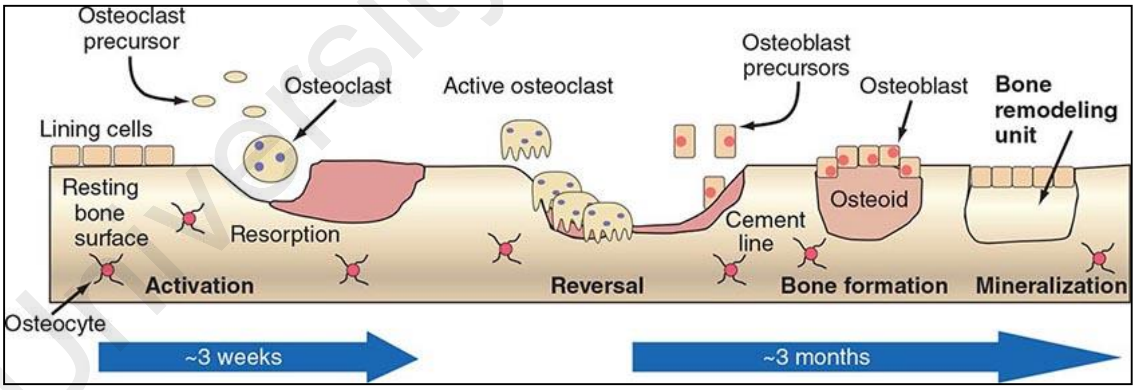


Figure 2.7: Schematic representation of bone remodeling. The cycle of bone remodeling is carried out by the basic multicellular unit (BMU), which consists of a group of osteoclasts and osteoblasts (Bringhurst et al., 2005).

2.8.1 Bone remodeling during sutural expansion

Studies on animals have characterized the idea of modeling of sutural bone under orthopedic stresses. Mechanical development of the midpalatal suture brings about the necrosis of the connective tissue inside the suture, trailed by vascular intervened wound healing reaction to re-establish osteogenic potential (Chang et al., 1997). Superficial infusion of the endothelial cell growth factor rhECGF improved angiogenesis in extended sutures however not in non-extended control sutures. DNA marking and histomorphometric investigation of multiplying cells in the healing blastemal showed that para-vascular pericytes are the source of osteoblasts in an extended suture (Chang et al., 1996; Chang et al., 1997). Following the healing reaction, the vascular induced osteoblast histogenesis was identical to the physiologic mechanism (Roberts&Morey, 1985) and orthodontic tooth movement (Roberts et al., 1982). Expansion of nasal sutures in rabbits, using implant placed endosseous as anchorages (Parr et al., 1999), gave critical data on the kinetics of sutural development. Like the PDL, sutures have the capacity for anabolic and catabolic modeling, to take into account 3-dimensional adjustment of craniofacial skeleton under applied stresses (Parr et al., 1997; Roberts, 2000).

2.8.2 Local regulation of bone remodeling during sutural expansion

At the point when stress is loaded onto the midpalatal suture, the force is changed into a biological signal that outcomes in a progression of biological events, for example, angiogenesis, chondrocyte hypertrophy, skeletal network arrangement, ligament and other tissue resorption, and matrix calcification. At last, mechano-transducted osteogenesis happens and the maxillary expansion occurs (Ma et al., 2008).

There are two sorts of osteogenesis, intramembranous and endochondral ossifications. A few examiners recommend that intramembranous ossification is essential to midpalatal suture development (Kobayashi et al., 1999). In light of HE staining and immunohistochemical investigation of Proliferating cell nuclear antigen (PCNA), it was discovered that following exposure to mechanical loads, osteoblasts multiplied between the ligament and periosteum. This ligament was slowly assimilated and replaced by bone and connective tissue, demonstrating that intramembranous ossification by osteoblasts from the palatal periosteum assumes a vital part in maxillary expansion (ME). What's more, utilizing Masson's staining, it was found that extensive skeletal matrices adjacent the suture ligament, proposing that endochondral ossification adds to osteogenesis in the beginning period of ME. In this way, the two sorts of osteogenesis take part in the tissue remodeling process during ME (Ma et al., 2008).

A few proteins, which were seen to change following introduction to the mechanical stress, merit extra investigations. To start with, osteoprotegerin (OPG) is a dissolvable "decoy" receptor that binds Receptor Activator for NF-kB Ligand (RANKL) and keeps it from binding to Receptor Activator for NF-kB (RANK) (Dobnig et al., 2006). This adequately restrains RANKL-intervened osteoclast development. The harmony between the osteoclast-advancing RANKL and the osteoclast-hindering OPG can direct the number and action of osteoclasts. Annexin A2 (Annexin II, ANXA2) is a member from the calcium-dependent phospholipid-binding protein family. It directs cell development and is engaged with signal transduction pathways. It is likewise critical for alkaline-phosphatase action in bone and is related with osteoblast mineralization (Gillette & Nielsen-Preiss, 2004). Parathyroid hormone (PTH) is delivered solely by the parathyroid organs and is transported to target tissues by dissemination through blood. The anabolic activity of PTH on cortical and cancellous bone has been approved (Jiang et al., 2003). The high PTH levels in ME tissues might be related with high local vascular thickness and additionally a rising concentration of serum PTH. Elongation factor 2 (EF 2) has a place with the G-protein superfamily and catalyzes the translocation phase of translation elongation after peptide bond development happens. It adds to the differentiation of chondrocytes to the hypertrophic stage, which is basic for endochondral ossification (Jefferies et al., 2000). This presence proposes cooperation of endochondral ossification in osteogenesis during ME. Cartilage glycoprotein 39 (GP-39) is created by various sorts of human cells, including macrophages, chondrocytes, neutrophils, and synovial cells (Aigner et al., 2001). It is highly found in osteoarthritic synovial fluids and osteophytic tissues and might be a more precise marker of chondrocyte initiation in the pathological process. This protein may take part in endochondral ossification and matrix resorption. These five proteins were all up-regulated in the beginning period of ME, and at any rate, some of them might be in charge of the impacts of ME (Ma et al., 2008).

2.9 Methods for bone tissue analysis

2.9.1 Dental radiographs

Periapical (PA) and orthopantomographic (OPG) radiographs are the main decision analytic clinical instruments in dentistry. PA radiographs with unrivaled resolution and sharpness give significant data to assessing the sum and patterning of trabecular bone structure (Whaites & Drage, 2013). Trabecular visibilities were accounted as higher on PA radiographs (Couture et al., 2003), along these lines improving its potential in trabecular imaging studies (Pham et al., 2010; Ribeiro-Rotta et al., 2011).

Bone characterizations are utilized to think about bone quality on PA pictures. A visual index was proposed in 1996 to simply classify trabecular systems on PA radiographs (Lindh et al., 1996). This index categorized trabecular patterns as per the intertrabecular spaces (little or substantial) and level of trabeculation (thin or thick) (Pham et al., 2010). In any case, these subjective systems remain in part approved (Ribeiro-Rotta et al., 2011). Then again, OPGs have additionally been utilized to survey trabecular structure (Watanabe et al., 2007). However, this technique applies the rotational principles that structures not centered in the focal trough are not sharply imaged. The formation of geometrical distortion, magnification and loss of information are thus commonly observed artefacts in OPG radiographs (Ibrahim et al., 2013). Furthermore, the diminished resolution of OPG corrupts its capacity in recognizing fine trabeculae

(Bollen et al., 2001). In this way, its applications in trabecular appraisals are less ideal than PA radiographs (Pham et al., 2010).

Irrefutably, using dental radiographs for surveying trabecular microstructure is a quick, moderately safe and helpful technique to apply in the jaws (Ibrahim et al., 2013). The complex shapes and structure of trabecular bone can be figured by performing fractal dimension (FD) investigation on 2D pictures, for example, periapical and OPG radiographs (Bollen et al., 2001). Current investigations on 2D FD examination of trabecular microarchitecture parameters (porosity, network, and anisotropy) are accounted for to be enough similar to that of 3D FD technique (Pothuaud et al., 2000). FD investigations and figuring of trabecular structures require a few complex steps (Jonasson et al., 2007). In addition, the general reproducibility of the projection strategies stays as hostile issue that requires further examinations.

2.9.2 Magnetic resonance imaging

MRI is a non-ionizing and non-invasive system, which applies high magnetic fields, transmission of radio-frequency waves and location of radio-frequency signals from energized hydrogen protons. Trabecular bone is loaded with bone marrow that contains free protons and creates a solid MR sign (Genant & Jiang, 2006). Fat and water protons in the marrow tissue are portrayed as negative picture. Since trabecular structure can't straightforwardly be pictured, this system utilizes picture handling to alter the negative picture (Ito, 2011). In spite of enhancing the trabecular structure evaluation, the quality of the acquired MR pictures is to a great extent impacted by the pulse arrangement, echo time, field strength and signal to noise proportion (SNR). Furthermore, the estimations are influenced by the image processing algorithms, chosen threshold values, complex investigations and elucidation of the pictures (Celenk & Celenk, 2010).

Moreover, the accessibility and availability of MRI machines for the dental professional stays limited (Ibrahim et al., 2013).

2.9.3 Computed tomography (CT)

Imaging technological advancements give enhanced opportunities to assess anatomical, morphological, physiological and clinical parameters. Imaging system and administrators have been developed to enable incredible spatial determination for the physiological and utilitarian properties of focused tissue, including tissue thickness, tissue volume, cell proliferation and oxygenation. One of these imaging developments is computed tomographic system (CT). CT is a three-dimensional radiographic imaging strategy. The picture arrangement process begins with the receiving of continuous radiographic projections caught over an angular position around the object. The cross-sectional field of perspective is changed using developed computational procedures relying upon radon projection speculation (Feldkamp et al., 1984). Like basic radiography, the reproduced picture's intensity represents the local radiographic constriction: a material property related to the objects' electron density (atomic number and mass thickness). The distinction among delicate and mineralized tissue in CT is high, as a result of the relative electron-dense inorganic component (calcium hydroxyapatite) of the bone matrix (Berger et al., 2005). Since the logarithm of the measured absorption scales straightforwardly with the length of material the beam has penetrated, simultaneous quantitative estimations of bone density are possible. Adjustment of gray scale straight constriction to BMD is refined by imaging reference phantoms containing objects with known hydroxyapatite fixations (Burghardt et al., 2008; Faulkner et al., 1993).

2.9.4 Cone beam computed tomography (CBCT)

CBCT systems were produced in the1990s, whereas in 2001, CBCT was presented as a 3D imaging methodology. The uses of CBCT in assessing bone quality are as yet confined on bone density evaluation (Araki & Okano, 2013; Naitoh et al., 2010). However, a study on assessing bone microstructure portrayed CBCT as a promising methodology for evaluating trabecular bone (Dos Santos Corpas et al., 2011). Bone parameters such as trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp) at mandibular condyle were also successfully evaluated by CBCT at a resolution of 125µm coupled with image processing (Liu et al., 2007).

The visibility of small anatomical structures with CBCT is to a great extent impacted by the field of view (FOV) and scan setting determination (Loubele et al., 2014). Visibility of trabecular microstructure is essentially dictated by the picked voxel size and SNR in addition to image artifacts (Moin et al., 2014). In CBCT, voxel size and slice thickness, spatial and contrast resolutions differ as for machine sort, FOV and scan settings (Loubele et al., 2014; Moin et al., 2014). Furthermore, a few image artifacts particular to CBCT technology could impact the effective system resolution, which could be lesser than the nominal system resolution expressed in voxel size alone. It has been beforehand expressed that the exactness of 3D estimation of anisotropic trabecular structure can be enhanced by performing *in-vivo* instead of *in-vitro* examination (Fyhrie, 2004). In this regard, the utilization of CBCT could demonstrate interest. As the need to assess the measure of bone formation after maxillary expansion has significantly increased, CBCT probably to be approved as a non-invasive method for evaluating bone microstructure with taking in considerations to be balanced by the potential risks of exposure to ionizing radiation.

2.9.5 Micro-computed tomography

The improvement of dedicated imaging systems for animals and, specifically, the CT have changed the utilization of animals models in musculoskeletal research, turning into the highest quality level for evaluation of bone morphology and micro architecture in animal models (Badea et al., 2008; Schambach et al., 2010). While histomorphometric examinations has been widely utilized as the principal standard for researching bone architecture, the revelation of 3D imaging systems, for example, CT have provided an exact noninvasive tool for straightforwardly measuring bone architecture. Unquestionably, since the introduction of clinical CT, the examination of small animals for research utilizing purpose built CT has immediately progressed giving great resolution, quick reconstruction and assessment protocols for preclinical applications (Bouxsein et al., 2010; Holdsworth & Thornton, 2002).

Micro CT utilizes X-ray attenuation images created at numerous viewing angle to recreate a 3D portrayal of the imaged object, describing the spatial distribution of the material density (Rüegsegger et al., 1996). At present micro CT scanners can provide high resolution with an isotopic voxel size of as low as a couple of micrometers (down to 5 μm; albeit new era on Nano-scanners can go down below 1 μm) (Wachsmuth & Engelke, 2004). There are basically two diverse micro-CT construction systems, one type in which the analyzed object is set in the middle and the X-ray detector and radiation source is mounted in a gantry that turns around it; in this system, the geometrical magnification is well characterized by the source-detector distance. This set-up is frequently used for animal models. In the second sort of CT scanner, the object is pivoted inside the course of the X-ray beam and the set-up grants the free positioning of the object between the detector and the source, permitting the modification of the magnification level. This second system is all the more frequently used in ex vivo custom-built systems. There are likewise contrasts on the beam geometry of the X-ray source utilized. Pictures can be acquired by utilizing either a fan-shaped beam in which information are procured through dynamic acquisition plane by plane or by a cone beam - this is also called 'volume-CT' (Feldkamp et al., 1984) where the scanned subject is captured totally (in light of the pivotal degree of the CT field of view) in one rotation, accelerating the imaging procedure. Moreover, micro-CT systems can be fixed with a flat panel based detector system with slip ring technology that licenses for fast data accumulation (Du et al., 2007). Lastly, micro-CT examination is fit for giving quantitative information through reproduction of virtual transversal areas of tissue objects; this constitutes a three-dimensional structural investigation (3D). Micro-CT is a strategy with high reproduction, potential ability and non-destructive attributes (Ibrahim et al., 2013). Also, the mineral substance can be mapped by recreating micro-CT scans indicating bone morphology modifications (Meleo et al., 2012).

2.9.6 Histological analysis

Many of the routine trichrome, hematoxylin/eosin (H&E), toluidine blue and other stains have been used for investigation of bone and ligament in paraffin sections. The fundamental histological staining for the general evaluation of cell and tissue morphology and distribution is the H&E stain: H&E stains cell cores blue-purple and the cartilage matrix pinkish with a somewhat blue aspect in the territories of a high proteoglycan content (An & Freidman, 1998).

Histological assessments and characterization of bone tissues are normally done under a light microscope. Contingent upon the specific circumstance, either or both might be utilized. Descriptive histology is utilized to give a general image of the tissue of interest, including the morphology, structure, and course of action of cells or matrix. Scoring systems are regularly outlined with a specific end goal to semi-evaluate the components of interest. For instance, full bone development in a defect is scored as 3, moderate bone development as 2, mild bone development as 1, and no bone as 0, and at times are presented as percentages. The information is analyzed utilizing non-parametric analysis of variance (An & Freidman, 1998).

Histomorphometric examination has been performed utilizing histological sections. It is a system for quantitatively analyzing length (the surface of an implant), zone (trabecular bone region or repair tissue region), and the number of components of interest (trabecular number, vessel number, or cell number) (Parfitt et al., 1987). These parameters are the essential measurements which can be made by viewing two dimensional (2D) pictures. It is frequently hard to reproduce a 3D structure based on a solitary 2D picture on the grounds that the structures of most biological tissues, (for example, bone tissue) are anisotropic. Disregarding its impediments, 2D histomorphometric investigation remains a typical and helpful technique for analyzing the structural changes in trabecular bone, the callus arrangement in healing fracture sites, the repair tissues of bone or cartilage defects, the bone relation and ingrowth into implant surfaces (Kang et al., 1998; Parfitt et al., 1987).

In the last two decades, there have been critical advances in histomorphometric techniques, to such an extent that semi-computerized and robotized image investigations coupled to modern stereology programming have to a great extent substituted the manual systems (Malluche et al., 1982). Momentous advances in bone histomorphometry were made in the 1950's and 60's expected the two noteworthy revelations. In the first place, there was the appearance of plastic implanting embedding high quality histologic areas of mineralized bone (Frost, 1958). Second, there was the utilization of labeling fluorochromes, for example, tetracycline which join at the mineralization front, leading to a better understanding of the dynamic process of bone development (Frost, 1969). Generally speaking, bone histomorphometry is a capable instrument for the evaluation of bone metabolism providing data that is not accessible by any other investigative approach. Furthermore, it gives priceless data on skeletal safety of new pharmacological interventions in clinical trials (Kulak & Dempster, 2010). Besides, histomorphometric examinations are viewed as the best quality level for the assessment of trabeculae and the cortical bone crest. They may give data on cellularity and dynamic indices of bone remodeling (Romão et al., 2015).

There are four primary strategies for histomorphometric information collection: The first method requires the utilization of a reticule imprinted with a grid fitted into the eyepiece of a microscope, hereafter referred to as the "point count method". This technique has been utilized as a part of research settings with varying types of grids (Epker & Frost, 1964; Stout, 1986; Stout & Paine, 1992), and it was all the more commonly utilized as a part of bone tissue analysis. Two of the strategies requiring image software have been utilized beforehand: utilizing a flatbed scanner (Peck & Stout, 2007) and taking overlapping pictures utilizing imaging software and compiling them with Adobe Photoshop® (Adobe Systems Incorporated, San Jose, CA) (Agnew & Stout, 2012). While the fourth strategy including the utilization of an advanced camera with extension tubes to increase the picture magnification without including additional glass. A metric ruler was incorporated into each picture with the goal that the pictures could be calibrated (Stewart et al., 2013). It was discovered that each strategy showed a determination of advantages and downsides varying from the cost of the equipment to the time required for arrangement and information gathering. In spite of potential downsides of every technique, in the hands of an experienced and well-trained scientist, every strategy can give important and legitimate results (Stewart et al., 2013).

2.9.7 Immunohistochemical analysis

Immunohistochemistry is valuable techniques for specialized bone, cartilage, tendon and ligament studies. In immunohistochemistry, organic substances of interest are localized by the precise connection of a complex or label which in this manner can be pictured in the cell or tissue of interest. Visualization can be either by bright microscopy or via UV microscopy using a fluorescent coupling agent. The visible product binds by the attraction between immunogen (antigen) and immunoglobulin (antibody). For orthopaedic research purpose, the specimens are typically either fresh frozen and sliced on a cryostat, or paraffin embedded tissue (An & Freidman, 1998). The two phosphohydrolases with special relevance are alkaline phosphatase (ALP), an ectoenzyme present in the osteoblast and in matrix vesicle membranes, and tartrate-resistant acid phosphatase (TRAP), a lysosomal enzyme whose localization gives a delicate technique for osteoclast characterization (Kulak & Dempster, 2010).

Bone specific proteins, for example, ALP and osteopontin (OPN) assume imperative parts in the cooperation between bone cells and the extracellular matrix (ECM). ALP played one of the key roles during osteogenesis. ALP has turned into the marker of choice while evaluating the phenotype or formative development of mineralized tissue cells. It is an imperative part in hard tissue development, profoundly expressed in mineralized tissue cells, and increased ALP action showed increased new bone arrangement (Golub & Boesze-Battaglia, 2007). It has been detected early expression of ALP which demonstrated the change towards a separated osteoblastic phenotype (Nguyen et al., 2015).

OPN is a non-collagenous multifunctional glycoprotein routinely present in mineralized tissues (Mckee & Nanci, 1996), which is believed to assume a vital part in cell reactions to mechanical stimulus. OPN seems, by all accounts, to be fundamental for mechano-stimulation of osteogenesis in the developing cranial suture. Then again, mechanical stimulus is known to incite and additionally increment the release of OPN in osteoblasts and osteocytes (Terai et al., 1999; You et al., 2001). These examinations suggest that OPN may assume a necessary part in the osteogenic reaction to mechanical stresses. An immunohistochemical (IHC) study of OPN expression in expansion of sagittal suture in mice found that OPN expression was enhanced during the bone formation and that OPN would be one of the positive components for the bone development under mechanical stresses (Morinobu et al., 2003). Different studies assessed OPN expression in formation of rodent mandible, they revealed different degrees of OPN expression by pre-osteoblasts, osteoblasts, and osteocytes (Pinero et al., 1995). Besides, this study noticed a dynamic increment in OPN detection in the membranous bone matrix that accompanied the development of the new bone. Different investigations have noticed a biphasic example of OPN expression corresponding to proliferation and differentiation of osteoblastic cells during bone formation (Aubin et al., 1995; Stein & Lian, 1993). From these investigations, we suggested that OPN assumes a fundamental part in the mechanical stimulation and maintenance of quick bone formation, and its expression during sutural extension may offer knowledge to the impact of different mechanical stimulus.

2.10 Animal model for sutural expansion

The nature of sutural changes because of orthopedic expansion forces can be discovered only in experimental animal studies. While the monkey (Gardner & Kronman, 1971) and the cat (Debbane, 1958) have maxillary sutures comparable in many perspectives to that of human and have been utilized as a part of maxillary expansion experiments, the ideal animals with which to acquire a clear picture of bone and suture changes under stress are the rabbit and the rodents (Storey, 1973). Helical torsion springs applying horizontal force to the upper incisor teeth might be utilized to actuate palatal expansion in these creatures. Early investigations indicated obvious contrasts in bone and suture changes, related with the use of various degrees of force to the upper incisor teeth of these creatures (Storey, 1955, 1973). In such species, premaxillae are moved horizontally around a focal point of pivot superior than the palatal vault, so the best rate of division happens at the oral aspect and the slowest, at the nasal aspect of the suture.

Most previous studies evaluating sutural expansion in New Zealand white rabbits have used the midsagittal suture. A 2010 study by Liu et al. found that continuous forces provided by nickel titanium coil springs were more effective for sutural expansion than intermittent forces (Liu et al., 2010). Their study used a MSI-supported expansion device and light continuous forces on younger animals. A 2011 follow-up study evaluated force levels for sutural expansion by applying variable forces 0, 50, 100, and 200 g. Their results showed that sagittal sutural widths in young rabbits increased by 0.6, 3.2, 5.1, and 6.2 mm, respectively (Liu et al., 2011). From prior studies, the midsagittal sutures of rabbits were found to be an important animal model for palatal expansion and also this model allowed for some extrapolation of results (Liu et al., 2011; Nunamaker, 1998; Pulver et al., 2016).

2.11 Summary

MTD are frequently encountered clinically and managed with tooth-borne RME. Orthodontic treatment using tooth-borne RME in adult patients is often associated with minimal skeletal expansion, extrusion of posterior teeth, inability to open the palatal suture and/or treatment relapse. An alternative approach is to apply expansion forces directly to the mid-palatal suture with bone-borne RME by the using of miniscrew implants. These bone-borne RME appliances produced greater orthopedic effects and fewer dento-alveolar side effects when compared to tooth-borne ones. The efficacy of bone-borne RME is affected by several factors including the rate of activation. Clinical activation protocols for tooth-borne RME may not be applicable to bone-borne ones. As there is currently no consensus on conventions for bone-borne RME, expansion protocols for these appliances warrant investigation. In addition, the quality and quantity of bone formation are dependent, albeit partially, on the rate of sutural expansion. While higher expansion rate has been associated with greater sutural separation, the exact nature of this association, as well as the maximum instant expansion possible without compromising sutural bone formation, has not been established.

The use of direct forces with the maximum instant expansion to expand mature midpalatal sutures can, however, lead to high sutural stresses and variable degrees of pain sensation. To reduce these high sutural stress and pain sensation, SARME were used to facilitate transverse maxillary expansion in mature patients. The midpalatal suture has been reported to offer the greatest resistance to maxillary expansion. Transcending bony resistance at the midpalatal suture is essential for success maxillary expansion in adult patients. In recent years, many methods of making surgical procedures for SARME simpler, safer and more predictable were explored including the use of piezoelectric corticotomy. The latter significantly reduces traumatic side effects, operation site bleeding, procedural and healing time associated with conventional rotary protocols. The precision of this tool allows clean, smooth and exact geometries during surgery. Therefore, several clinical applications of piezoelectric surgery in SARME and Le Fort I osteotomy was published. However, there have been no studies evaluated the outcome of piezoelectric sutural corticotomies on SARME.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study design

This was a randomized post-test comparison study to examine the relationship between the amount of the instant (initial) expansion, sutural separation and new suture bone formation for the accelerated bone-borne sutural expansion. This study also aimed to evaluate the effects of sutural piezoelectric corticotomy on the amount of sutural separation and new suture bone formation for the accelerated bone-borne sutural expansion.

3.2 Ethical approval

The study was approved by the Faculty of Medicine Institutional Animal Care and Use Committee (FOM IACUC), University of Malaya (UM) (2015-16/006/DENTAL/R/ASH) and all animal work was performed according to the standards specified by the "institutional animal care and use" committee, UM, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Date of approval was on 16th October 2015 (appendix C).

3.3 Animal recruitment

3.3.1 Sample size calculation

New Zealand White male rabbits (n=26, age= 20 to 24 weeks; body weight ~ 3.0-4.0 kg) were selected for the study. Sample size calculation was based on the Resource Equation method (E) (Festing, 2006; Festing & Altman, 2002). "E" can be measured by the following formula: E = Total number of animals − Total number of groups. According to this method, a sample size which keeps E between 10 and 20 can be considered as adequate (E was 19 in the present study). In addition, previous studies evaluating bone regeneration in the frontal bone of rabbits had used a sample size of 2 to 3 rabbits per group (Jayash et al., 2017; Puhar et al., 2016).

3.3.2 Animal selection and preparation

The rabbits were procured from a licensed farm by the animal experimental unit, UM. Upon arrival, the rabbits were examined, weighed and observed twice daily for two weeks by a trained animal laboratory staff till the conduct of the experiment for acclimatization. The clinical observation includes checking the activities (alert or not), pattern of movement (whether the animals walking on tiptoe or reluctant to move), respiration, hair coat, drinking/eating (well or not), eyes and nose (check for any discharge), feces (whether there is diarrhea or not). Environmental conditions were maintained as follows: Specific pathogen free with a temperature of 22°C ±3°, humidity of 55% ±10%, and a 12:12 light:dark cycle with lights on at 0700 and off at 1900. Animals were housed singly in cages (Techniplast, Buguggiate, Italy) with paper liner floor. The breeding programme was monomating with maintenance food (altromin 2023 diets) (Sterling Ascent, Penang, Malaysia) and reverse osmosis treated water. A completed Animal Research Reporting of *In-Vivo* Experiments (ARRIVE) guidelines checklist is included. Preoperative intramuscular ketamine 30 mg/kg ketamine (Troy Laboratories, Smithfield, Australia) and 3 mg/kg xylozine (Troy Laboratories, Smithfield, Australia) were first administered. The distraction sites were then anesthetized using Marcaine (2 mg/kg) (Abbott Laboratories, Illinois, USA) with 1: 200,000 epinephrine (0.005 mg/mL) (Troy Laboratories, Smithfield, Australia). General anesthesia was subsequently accomplished by a trained animal laboratory staff using 1-3% Isoflurane in a 2:1 oxygen/nitrous oxide mixture administered through special face masks.

3.4 Expansion procedures

3.4.1 Expansion procedures for the phase one

The phase one in this dissertation was conducted to answer the following research questions: 1) Is there any differences in sutural separation and bone formation between dissimilar bone-borne expansion protocols? 2) Is there an optimum instant sutural expansion for bone-borne sutural expansion beyond which bone remodeling and formation will be disrupted?

Modified expanders were fabricated by laser welding four 0.9 mm stainless steel 'U' loops to Hyrax expanders (Leone Spa, Firenze, Italy) as shown in the Figure 3.1A. The mucosa and periosteum at the distraction sites between the right and left limits of the orbital rims were reflected by doing two horizontal incisions to expose the midsagittal sutures and frontal bones (Figure 3.2). Then, pilot holes were drilled with a size-2 round burr with a low-speed hand piece (<600 rpm) and copious saline-solution irrigation. Four Dentos (Daegu, Korea) miniscrew implants (MSIs; 5.0 mm long 1.7 mm in diameter) (Figure 3.1B) were placed approximately on either side of the midsagittal suture with a manual driver. After that, the customized expanders were positioned across the midsagittal sutures (Figure 3.3) and secured with the miniscrew implants. The surgical flaps were subsequently closed and the rabbits were given intramuscular Kombitrim® 1 ml/10kg (sulfamethoxazole 200 mg and trimethoprim 40 mg) (Kela Laboratoria n.v, Hoogstraten, Belgium) post-operatively to prevent infection and meloxicam 0.2 mg/kg (Poly Car Labs, Gujarat, India) to minimize discomfort.

The rabbits with their bone-borne expanders were randomly divided into four groups (4 rabbits per group) and the Hyrax appliances were activated as follows: Group 1 (control), 0.5 mm expansion/day for 12 days (Akin et al., 2015); group 2, 1 mm instant expansion followed by 0.5 mm expansion/day for 10 days (Paley, 1988; Welch et al., 1998); group 3, 2.5 mm instant expansion followed by 0.5 mm expansion/day for 7 days, and group 4, 4 mm instant expansion followed by 0.5 mm expansion/day for 4 days. Thus, a total expansion of the Hyrax appliances of 6 mm was applied for all groups Instant expansion refers to the amount of expander activation (mm) immediately after closing the surgical flaps. Upon completion of the active expansion, the screws on the Hyrax appliances were fixed with a light-cured acrylic and left passive. After 6 weeks of retention, sutural separation and new bone formation was assessed by micro-CT. Then the rabbits were euthanized by a high dose (90 mg/kg) of phenobarbitone (Figure 3.4).

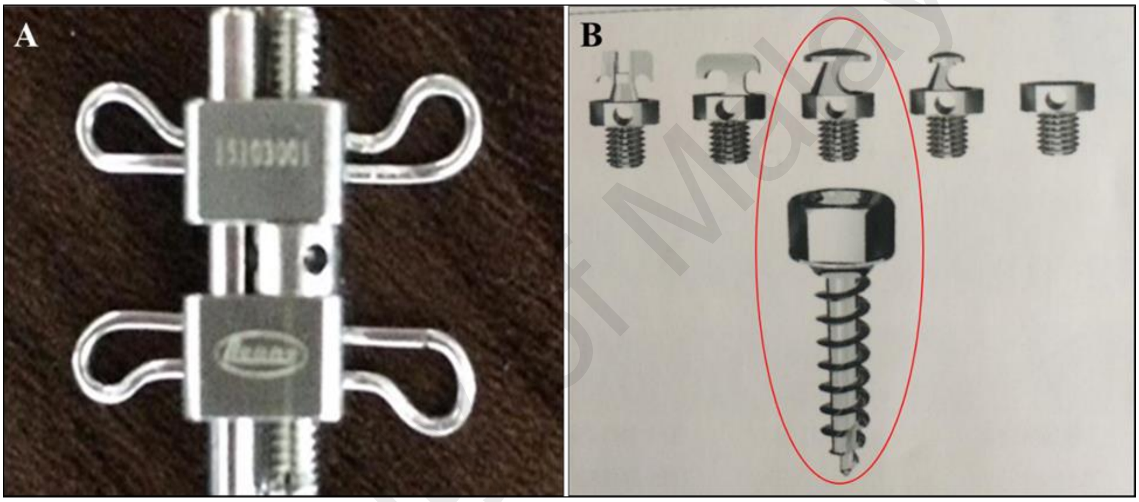


Figure 3.1: A) A modified hyrax expander with 4 coils (Leone Spa, Firenze, Italy), B) Miniscrew implants (Dentos Inc., Daegu, Korea).

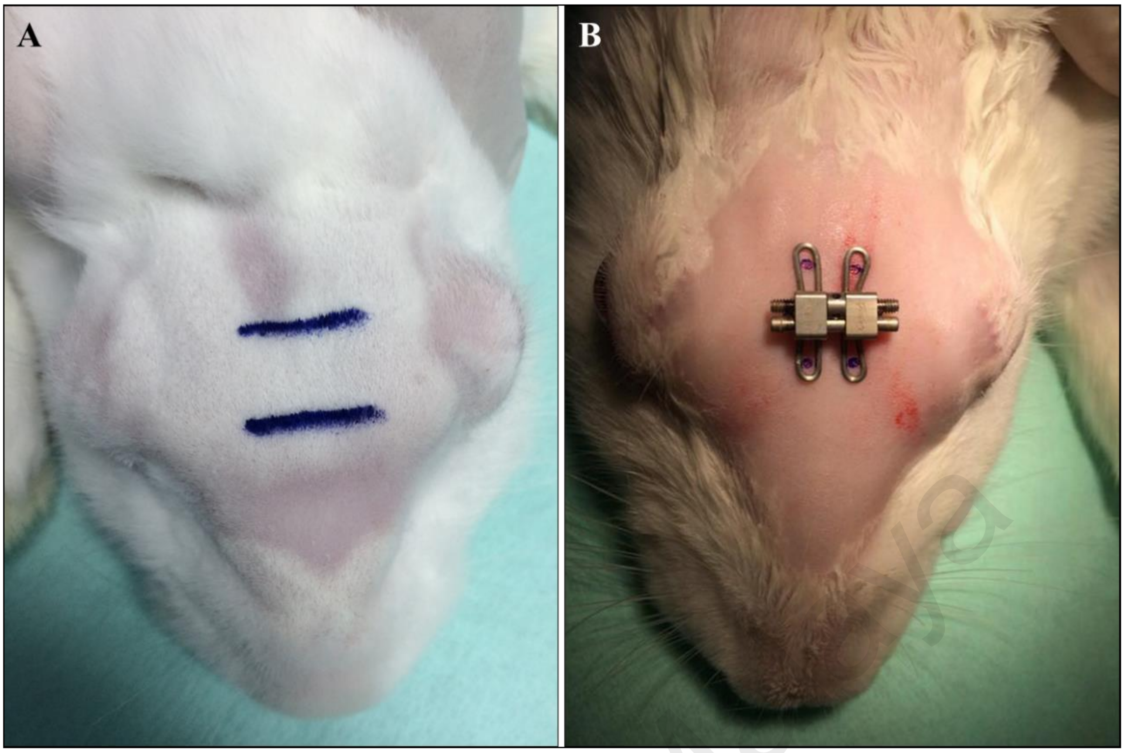


Figure 3.2: The images showing (A) the proposed positions for the surgical incisions (blue lines), (B) the proposed positions for the miniscrew implants (blue points).

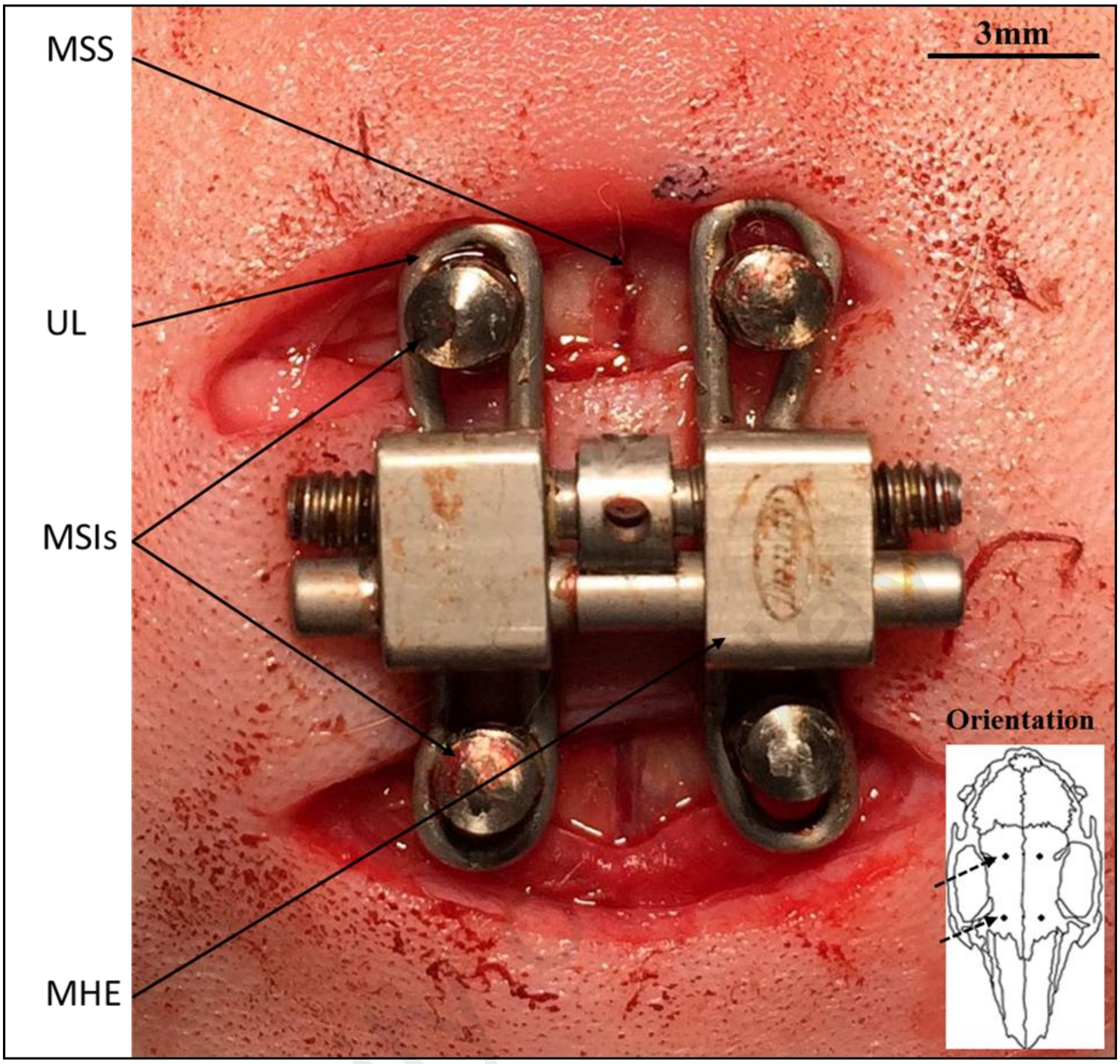


Figure 3.3: The modified hyrax expander secured with miniscrew implants immediately after 0.5 mm expansion (Group 1 of phase one). MSS, midsagittal suture; UL, U loop made from 0.9mm stainless steel wire; MSIs, miniscrew implants (5.0 mm length, 1.7 mm in diameter); MHE, modified hyrax expander; the dotted arrows indicate the location of the miniscrew implants.

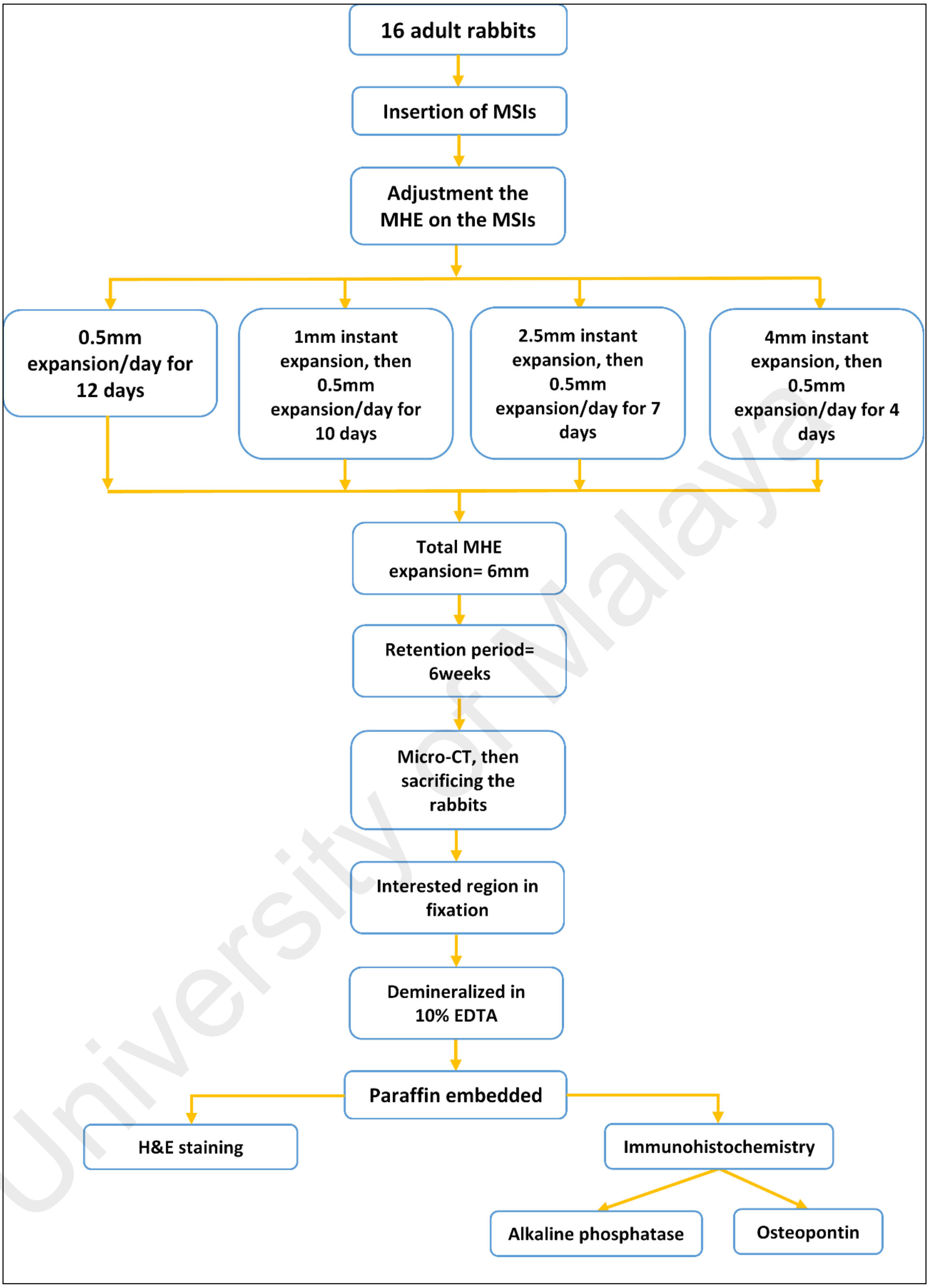


Figure 3.4: Research design for the phase one. MSIs, miniscrew implants; MHE, modified hyrax expander; Micro-CT, micro-computed tomography; EDTA, ethylenediamine tetraacetic acid; H&E, hematoxylin and eosin.

3.4.2 Expansion procedures for the phase two

The phase two in the present dissertation was conducted to answer the following research questions: 1) What is the effect of piezoelectric sutural corticotomies on the outcome of accelerated bone-borne sutural expansion? 2) Is sutural separation and new bone formation between continuous and discontinuous sutural piezoelectric corticotomies identical?

The rabbits in this phase were divided randomly into three groups according to the expansion technique (i) group 1 (n=4) accelerated bone-borne sutural expansion without corticotomy, (ii) group 2 (n=5) accelerated bone-borne sutural expansion with continuous piezoelectric sutural corticotomy, (iii) group 3 (n=5) accelerated bone-borne sutural expansion with discontinuous piezoelectric sutural corticotomy.

Briefly, after reflecting the skin and the periosteum midway between the right and left limits of the orbital rims, the midsagittal suture and the frontal bone were exposed. Then two types of corticotomies were performed straight on the midsagittal suture by using a piezoelectric device (Woodpecker DTE®, DS-II., Guangxi, China) and diamond surgical tip (model; US1, size; 4 mm, thickness; 0.5 mm) (Figure 3.5). While the expansion technique in the first group (control group), was without corticotomy, a continuous sutural corticotomy was done in the second group (20 mm long through midsagittal suture) and a discontinuous sutural corticotomy (4 mm cuts with 4 mm intermissions over 20 mm) was performed in the third experimental group. The 4 mm interval between the cuts was used as the size of the cutting tip is 4 mm. The piezoelectric cuts, which were 1.5 to 2 mm deep, were made on nearly the whole suture with selected portions left intact (inner surface of the suture toward the nose). Then, pilot holes were drilled with a size-2 round burr with a low-speed hand piece (<600 rpm) and copious saline-solution irrigation. Four Dentos (Daegu, Korea) miniscrew implants (MSIs; 5.0 mm long 1.7 mm in diameter) were placed approximately on either side of the midsagittal suture with a manual driver. After that, the customized expander was engaged in between the MSI heads (Figure 3.6). Then the surgical flaps were subsequently closed and the rabbits were given intramuscular Kombitrim® 1 ml/10kg (sulfamethoxazole 200 mg and trimethoprim 40 mg) (Kela Laboratoria n.v, Hoogstraten, Belgium) post-operatively to prevent infection and meloxicam 0.2 mg/kg (Poly Car Labs, Gujarat, India) to minimize discomfort.

Figure 3.5: Ultrasonic Piezo Surgery (Woodpecker DTE®, DS-II., Guangxi, China) with handpiece and tip.

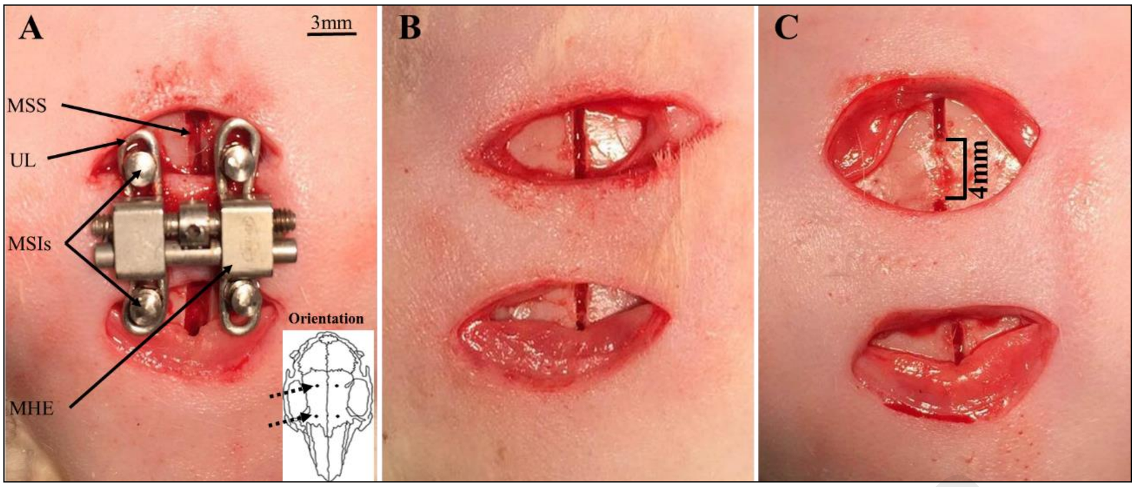


Figure 3.6: A) A modified hyrax expander secured with miniscrew implants, immediately after 2.5 mm instant expansion in the group 1, B) Group 2 immediately after continuous corticotomy, C) Group 3 immediately after discontinuous corticotomy. MSS, midsagittal suture; UL, U loop made from 0.9 mm stainless steel wire; MSIs, miniscrew implants (5.0 mm length, 1.7 mm in diameter); MHE, modified hyrax expander; the dotted arrows indicate the location of the miniscrew implants.

The activation of the modified expanders for the all groups was 2.5 mm instant expansion followed by 0.5 mm/day for 7 days. Thus, a total expansion of the Hyrax appliances of 6 mm was applied for all groups. Upon completion of the active expansion, the screws on the Hyrax appliances were fixed with a light-cured acrylic and left passive. After 6 weeks of retention, sutural separation and new bone formation were assessed using micro-CT. Then the rabbits were euthanized by a high dose (90 mg/kg) of phenobarbitone (Figure 3.7).

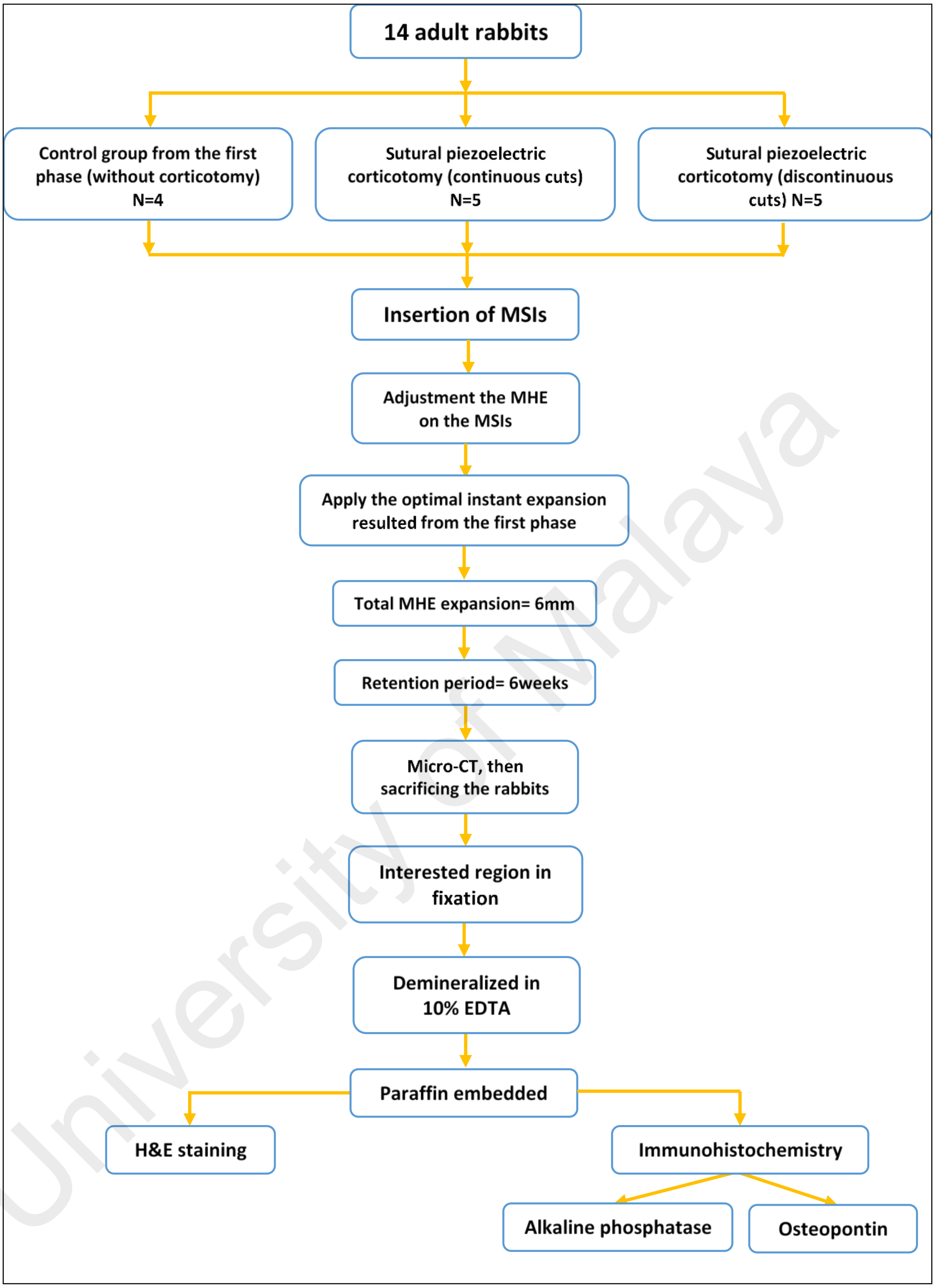


Figure 3.7: Research design for the phase two. MSIs, miniscrew implants; MHE, modified hyrax expander; Micro-CT, micro-computed tomography; EDTA, ethylenediamine tetraacetic acid; H&E, hematoxylin and eosin.

3.5 Micro-computed tomography imaging

Prior to micro-CT imaging, the rabbits were again anesthetized with ketamine and xylazine. Post-treatment scans of the distraction sites were obtained using high-resolution *in-vivo* X-ray micro-CT imaging (XtremeCT, Scanco Medical, Bassersdorf, Switzerland) (Figure 3.8). Serial tomographic images were acquired transverse to the midsagittal sutures at 60 kVp and 900 μA. One thousand projections were acquired per rotation with an integration time of 300 ms and a voxel size of 41 μm^3.

Figure 3.8: The image shows Scanco XtremeCT device used in the experiment. It is specially designed to perform a high-resolution peripheral quantitative computed tomography image.

An aggregate of 160 slices located between the anterior and posterior miniscrew implants were selected. A region of interest (ROI) was selected 3 mm from the midline (left and right) and applied to all samples (Figure 3.9A). Three-dimensional images of the ROI were automatically reconstructed from these 160 slices using the Scanco Micro-CT V6.5.3 software (Figure 3.9C). The grayscale images were smoothed by a Gaussian filter with a sigma value of 0.9 and support value of 1. The threshold value was set between 130 (lower value) and 1000 units (upper value) to discriminate the less dense newly formed bone and the denser old bone (Freeman et al., 2009) based on previous similar study (Lu & Rabie, 2003) and data from our pilot study. Bone volume fraction (BV/TV) at the suture was then measured using Scanco Micro-CT V6.5.3 software.

After segmentation of bone tissues using the above threshold values, a 3D color map of the suture tissue separation was generated. Separation maps of the suture tissue indicate maximum separation values with blue to red colors specifying increasing degrees of tissue separation (Figure 3.9B) (Hildebrand & Rüegsegger, 1997; Palmer et al., 2006).

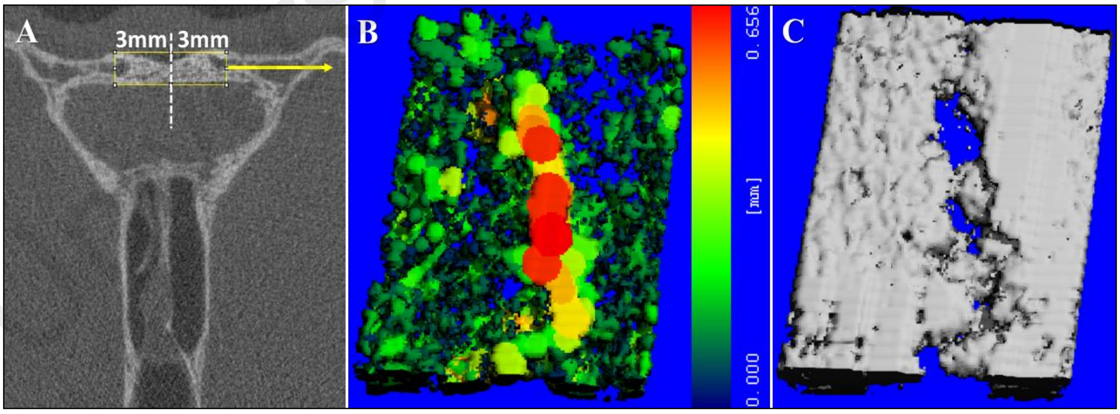


Figure 3.9: A) 2D image showing the regions of interest at the midsagittal suture 6 weeks` post retention, B) 3D map showing the degree of suture tissue separation, increasing from blue to red (blue indicates no tissue separation; red indicates maximum separation), and C) 3D segmentation showing the sutural space. The white dotted line (A) represented the midline of the suture. The ROI was 3 mm on either side of the dotted line.

To calculate the sutural space volume, micro-CT images were exported into Mimics Medical 17.0 imaging software (Mimics, Materialise, Leuven, Belgium). Lower and upper thresholds were set for all specimens between 224 to 1249 unit to separate soft tissue from bone tissue. As a result, the soft tissue was highlighted in yellow using so-called masks. The mask was cropped to select the ROI and used for all specimen. Region growing (computer-assisted tissue separation) and manual tissue deletion (using multiple slice editing tool) were employed to isolate the ROI (Regensburg et al., 2008). The software then calculated sutural soft tissue volume (sutural space volume) by means of voxel addition and reconstructed a 3D image (Figure 3.10).

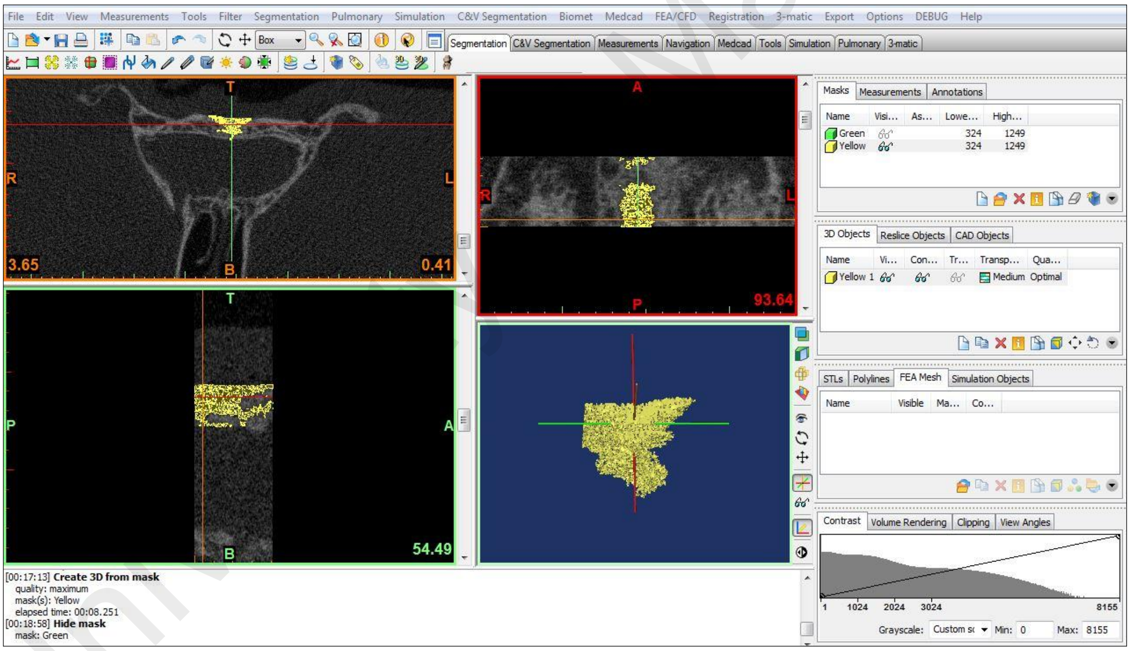


Figure 3.10: Overview of the axial, coronal, and sagittal micro-CT slices with the highlighted segmented tissue (yellow color indicates the sutural space volume), and the 3D reconstruction of the segmented tissue.

Amount of sutural separation was established with Radiant DICOM viewer version 3.4.1. The most anterior and posterior slice for the ROI were first ascertained to locate the bony outline of the sutures. Sutural separation was then calculated with the distance measurement tool (Figure 3.11) at the point equidistant between the outer surface

(OS) and inner surface (IS) of frontal bone and using an average of two separate readings. Intra-examiner reliability for mapping the ROI, sutural space volume and measuring suture separation were assessed by repeating the procedures blinded after two weeks. Reliability was evaluated using the Cronbach alpha test.

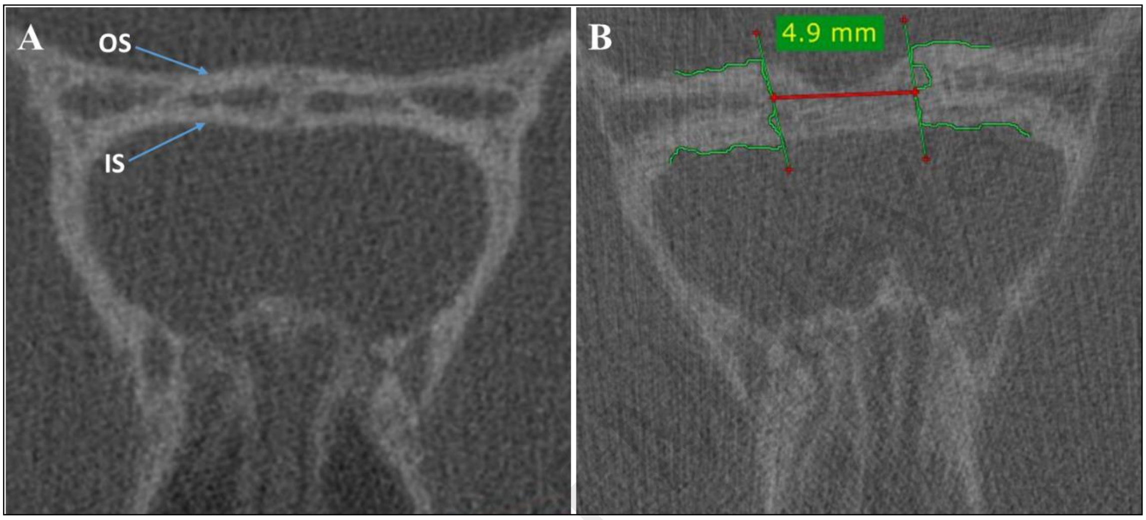


Figure 3.11: Method of measuring sutural separation at the midsagittal suture of rabbits (separation equidistant between OS and IS). (A) Pre-expansion. (B) 6 weeks' post-expansion. OS, outer surface (toward skin); IS, inner surface (toward nasal floor); 4.9 mm, the amount of sutural separation.

3.6 Histology and immunohistochemistry

After sacrificing the rabbits, a standardized area, including the midsagittal region and adjacent bone, was dissected, fixed with 4% paraformaldehyde prepared in phosphate buffer saline (PBS) for 48 hours at 4°C, washed scrupulously with running water and decalcified in 10% ethylenediamine tetraacetic acid (EDTA-2Na, pH 7.4) for 2 weeks, dehydrated with a series of ethanol solutions of increasing concentration (75%, 80%, 95% and then 100%) and embedded in paraffin. The paraffin blocks were sectioned coronally to 4 μm-thick slices in a microtome at room temperature and mounted on polarized glass slides. For each paraffin block, three slides were assigned for histology and another three for immunohistochemistry. All the above mentioned procedures were performed with the assistant of assistance of pathology laboratory technologists, Faculty of Dentistry, UM.

Histology specimens were stained with H&E and digitized with the Panoramic SCAN digital slide scanner (3DHISTECH, Budapest, Hungary). The histological images were subsequently assessed using the Panoramic viewer software version 1.15.3 (3DHISTECH, Budapest, Hungary) at 4 times magnification. The amount of sutural separation was determined at the upper, middle and lower parts of the lateral edge of each suture. The position of the 3 segments was defined by the incremental lines that separate old from newly formed bone. Distance between the two sides of the 3 suture segments was measured using the micrometer measuring tool in the software and averaged for each specimen and rabbit. The expanded suture with adjacent 2 mm of frontal bone yielded a total of 78 sections, from which 390 microscopic fields (four peripheral and one central) were obtained for the various experimental groups. Quantitative analysis was performed using the Image-Pro Express software (Media Cybernetics Inc., Bethesda, MD, USA) for Windows. A 48 points grid was then overlaid on each microscopic field to measure the amount of newly formed bone, blood capillaries and non-osteoid tissue in the sutures using point-counting method (Stewart et al., 2013). The number of points on the newly formed bone matrix, blood capillaries, and non-osteoid tissue were quantified and statistically analyzed.

For immunohistochemistry, the sections were deparaffinized and rehydrated through xylene and serial dilutions of ethanol (100%, 95%, 80%, and then 75%) in distilled water. The labeling was performed according to established procedures (Bondarenko et al., 2014; Gruber & Ingram, 2003) using the Dako REALTM EnVision Kit (K500711). Briefly, the sections were treated with the target antigen retrieval and citrate buffer for 20 mins at 100°C, blocked with 1% bovine serum albumin in phosphate-buffered saline (PBS) for 30 min at room temperature, and then incubated with the following primary antibodies: mouse anti rabbit alkaline phosphatase (ALP) monoclonal antibody (10 µg/ml, ab17973; Abcam, Cambridge, UK) and mouse anti rabbit osteopontin (OPN) monoclonal antibody (1:100, NB110-89062; Novus Biologicals, Cambridge, UK) for 1 hour at room temperature and ~90% humidity. The samples were next washed with buffer and incubated with secondary antibodies (Envision/ HRP detection) for 30 minutes at room temperature and ~90% humidity. Negative controls were attained by omitting the primary antibody step and repeating the afore mentioned procedures. The sections were counterstained with hematoxylin and digitized with the Panoramic SCAN digital slide scanner. Five fields from each section were captured at 40 times magnification and examined using the ImageJ software version 1.50i (Wayne Rasband, NIH, USA) for quantitative evaluation of immunohistochemical staining (Gentile et al., 2015; Jayash et al., 2017). The immunohistochemical index was automatically generated with the thresholding tool of the ImageJ software until all the stained areas were selected. Thresholding tool settings were standardized to allow for comparison between images. Inter-examiner reliability for measuring the amount of sutural separation, point-counting method and the intensity of labeling for immunohistochemistry were assessed by two blinded examiners.

3.7 Statistical analysis

All data were analyzed with the Statistical Package for Social Sciences 20.0 (SPSS for Windows, SPSS Inc., Chicago, USA). Normality testing was done using Shapiro-Wilk test. As data were not normally distributed, non-parametric Kruskal Wallis and Mann-Whitney U tests ($p<0.05$) were used to determine the significant differences in sutural separation, bone volume fraction, the various bone parameters, newly formed bone, angiogenesis, ALP and OPN markers between experimental groups. Post hoc analysis was performed with Bonferroni correction to confirm the results of multiple comparisons.

Spearman's rho correlations ($p<0.05$) were also performed to establish the associations between the amount of instant expansion & sutural separation, and the amount of instant expansion & bone volume fraction / new bone formation. Reliability was evaluated using the Cronbach alpha test ($\alpha \geq 0.7$).

CHAPTER 4: RESULTS

4.1 Animal status and rate of success

The animals did not show obvious signs of systemic illness throughout the study period. Mucosal infection, dehiscence, or other adverse effects were not observed in any of the rabbits. There were also no signs of distraction site infection or animal discomfort from using the modified expanders. No substantial changes in body weight were observed between the experimental groups during the expansion and retention periods (weight gained was ranging from 0.72 to 0.97kg).

The overall success rate of the miniscrew implants was 98.44% (63 out of 64) and 100% (56 of 56) for the phase one and two, respectively. Only one miniscrew implant was displaced in the phase one upon direct Hyrax activation and was due mainly to operator issues. The dislodged miniscrew implant was in group 1 and promptly replaced with a new one buccal to the original site. The miniscrew implant assisted bone-borne modified hyrax expanders with and without corticotomy successfully expanded the midsagittal sutures for all experimental groups.

Reliability testing revealed no significant difference in measurements between the two assessment periods with regards to micro-CT analysis and the two blinded examiners with regards to sutural separation, point-counting method and the intensity of labeling for immunohistochemistry (phase one: α= 0.946, 0.940, 0.920 and 0.960, respectively; phase two: α= 0.920, 0.890, 0.940 and 0.910, respectively) (appendices D, E, and F).

4.2 Micro-computed tomography analysis for the phase one

4.2.1 Sutural separation

Median values were 2.99, 3.12, 3.77 and 4.55 mm for anterior sutural separation, and 2.71, 2.78, 3.61 and 4.27 mm for posterior sutural separation for groups 1 to 4, respectively. Median values for average sutural separations were 2.84, 2.92, 3.69 and 4.41 mm for groups 1 to 4 respectively (Table 4.1). Paired comparisons showed statistically significant differences in sutural expansion between all groups. Groups 4, 3 and 2 had significantly larger sutural separation than the control group (group 1). Sutural separation for groups 4 and 3 were in fact 55.28% and 29.93% greater than group 1. Sutural separation for group 4 was significantly larger than groups 3 and 2, whereas group 3 had significantly greater separation than group 2 (Figure 4.1). Spearman's correlation test showed strong, positive and significant correlation (*r*=0.970, $p<0.01$) between sutural separation and amount of instant expansion.

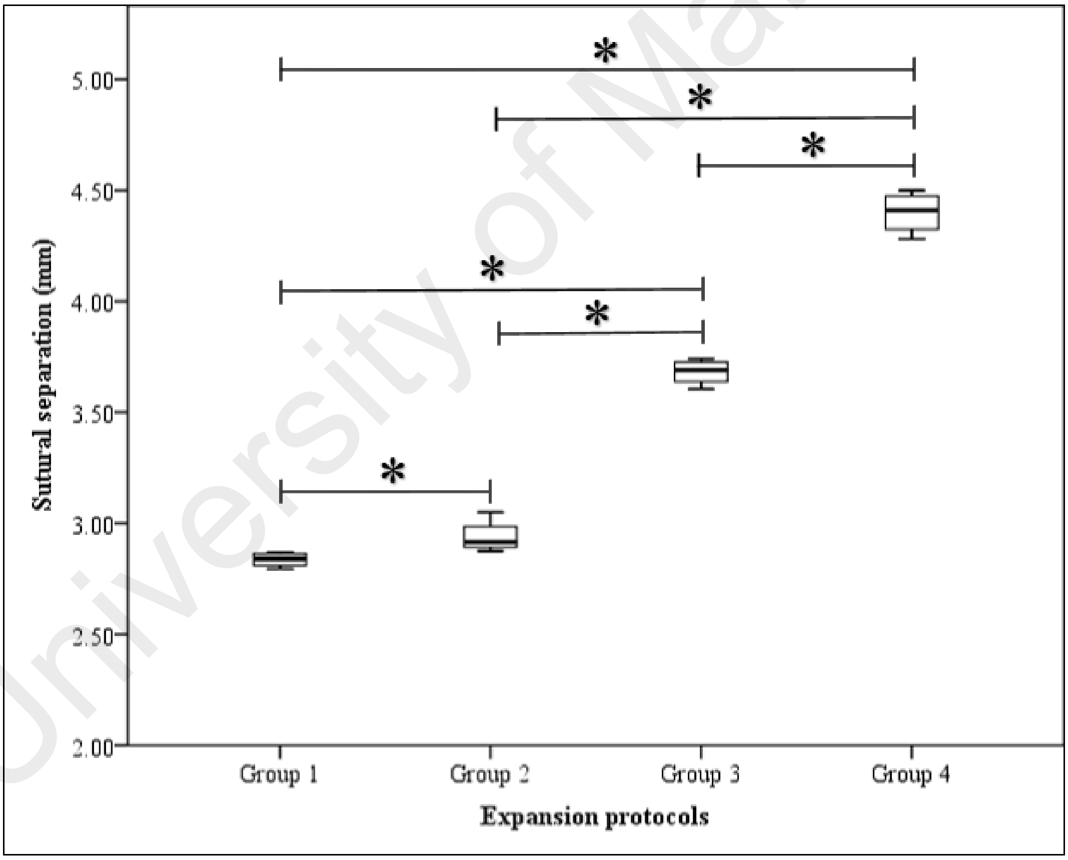


Figure 4.1: Median sutural separation (mm) for the various groups of the phase one with micro-CT at 6 weeks post retention. As instant expansion increases, the amount of sutural separation increases accordingly. Maximum sutural separation was observed in group 4 (4.41 mm) and the minimum in group 1 (2.84 mm). * indicates significant differences at p<0.05 (Mann-Whitney U test).

4.2.2 Bone parameters

Figure 4.2 shows 2D images for ROI, 3D color map, and 3D segmentation for the various groups of the phase one at 6 weeks post retention. We found that the amount of suture tissue separation (red color) was minimum in group 3 and maximum in group 4. Furthermore, the expanded sutures were clearly outlined and filled with a variable quantity of new bone formation that virtually closed the sutural gaps in groups 3, 2 and 1, while in group 4 was almost vanished. Median BV/TV, sutural space volume, and sutural tissue separation after 6 weeks of retention are also shown in Table 4.1. Group 4 had significantly lower BV/TV than all the other groups. Group 3 had significantly higher BV/TV than group 2 and group 1 (Figures 4.3). The amount of sutural space volume in group 4 was significantly higher when compared to the other groups, while in group 3, it was significantly lower than all other groups. Similarly, the amount of sutural tissue separation in group 4 was significantly larger than all other groups, while in group 3 was significantly smaller when compared to the other groups.

The relationship between BV/TV and sutural separation was found to be linear up to 3.69 mm (group 3). After this critical point, the relationship was inverted and BV/TV was decreased. In contrast, Spearman's correlation test showed strong, negative and significant correlation (r= -0.932, $p<0.01$) between BV/TV and the amount of sutural space volume.

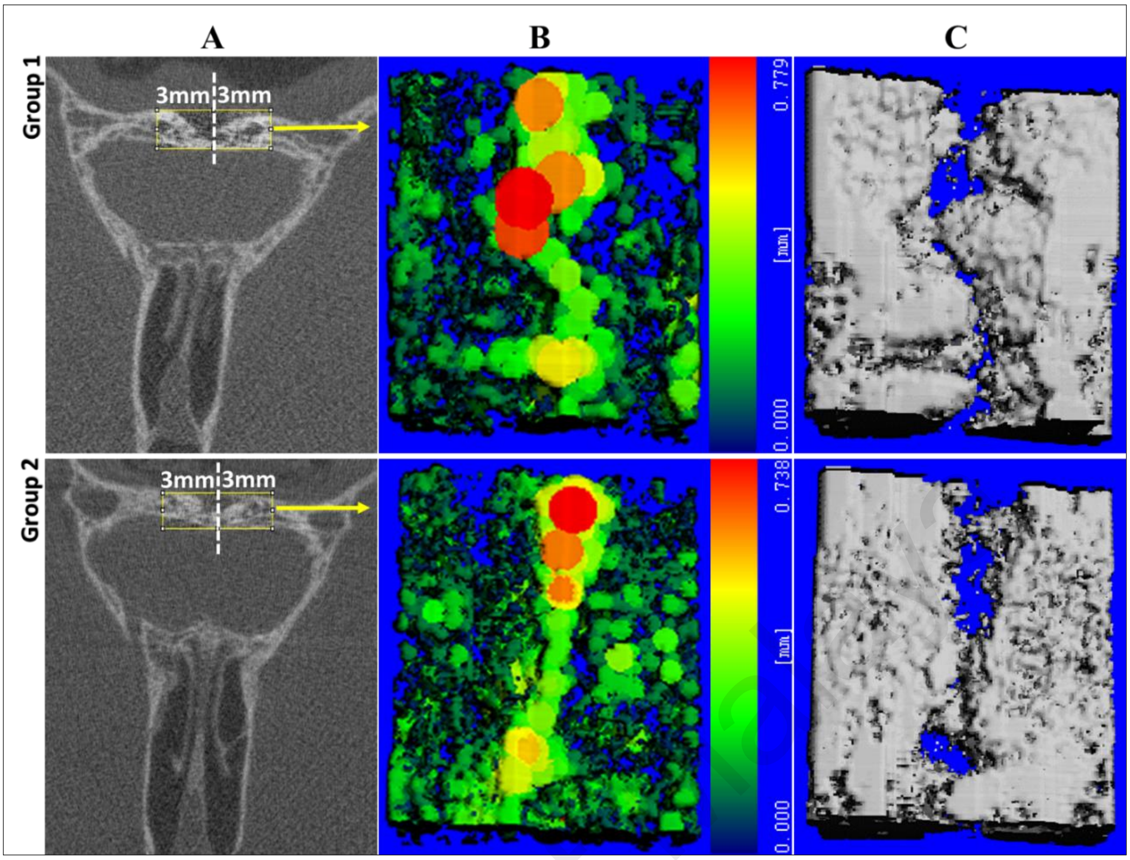


Figure 4.2: A) 2D image showing the regions of interest at the midsagittal suture 6 weeks post retention for the phase one, B) 3D map showing the degree of suture tissue separation, increasing from blue to red (blue indicates no tissue separation; red indicates maximum separation), and C) 3D segmentation showing the sutural space. The white dotted line (A) represented the midline of the suture. ROI was 3 mm from the right and left side of the suture.

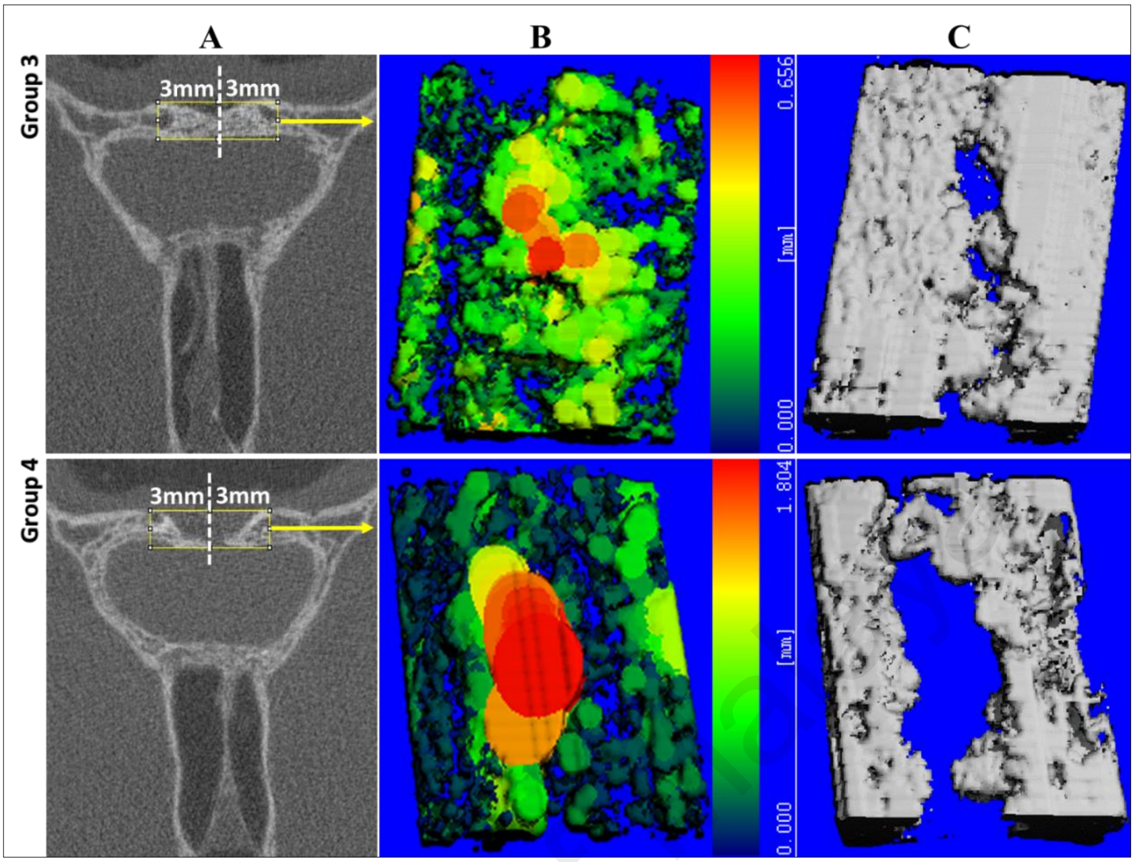


Figure 4.2: (Continued).

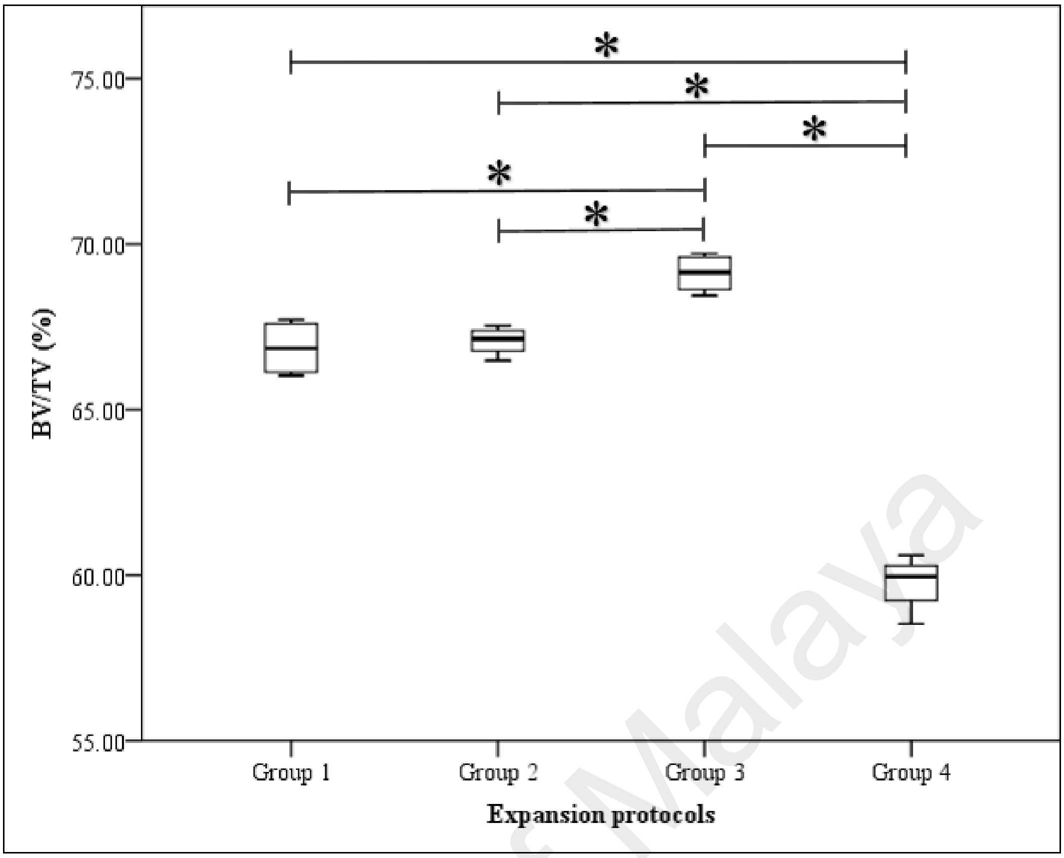


Figure 4.3: Median bone volume fraction changes % (BV/TV) for the various groups of the phase one with micro-CT at 6 weeks post retention. Maximum BV/TV was observed in group 3 and minimum in group 4. * indicates significant differences at p<0.05 (Mann-Whitney U test).

4.3 Histomorphometric and immunohistochemical analysis for the phase one

4.3.1 Sutural separation

Biometric analysis for the amount of sutural separation from histological sections showed median sutural separations of 3.05, 3.24, 3.97 and 4.57 mm for groups 1 to 4, respectively (Table 4.1). Paired comparisons showed statistically significant differences in sutural expansion between groups. Groups 4 and 3 had significantly greater sutural separation than the control group (group 1). While sutural separation for group 2 was larger than group 1, the difference was not statistically significant. Sutural separation for groups 4 and 3 were in fact 49.84% and 30.16% greater than group 1, respectively (Figure 4.4). Spearman's correlation test showed strong, positive and significant correlation (*r*=0.95, $p<0.01$) between sutural separation and amount of instant expansion.

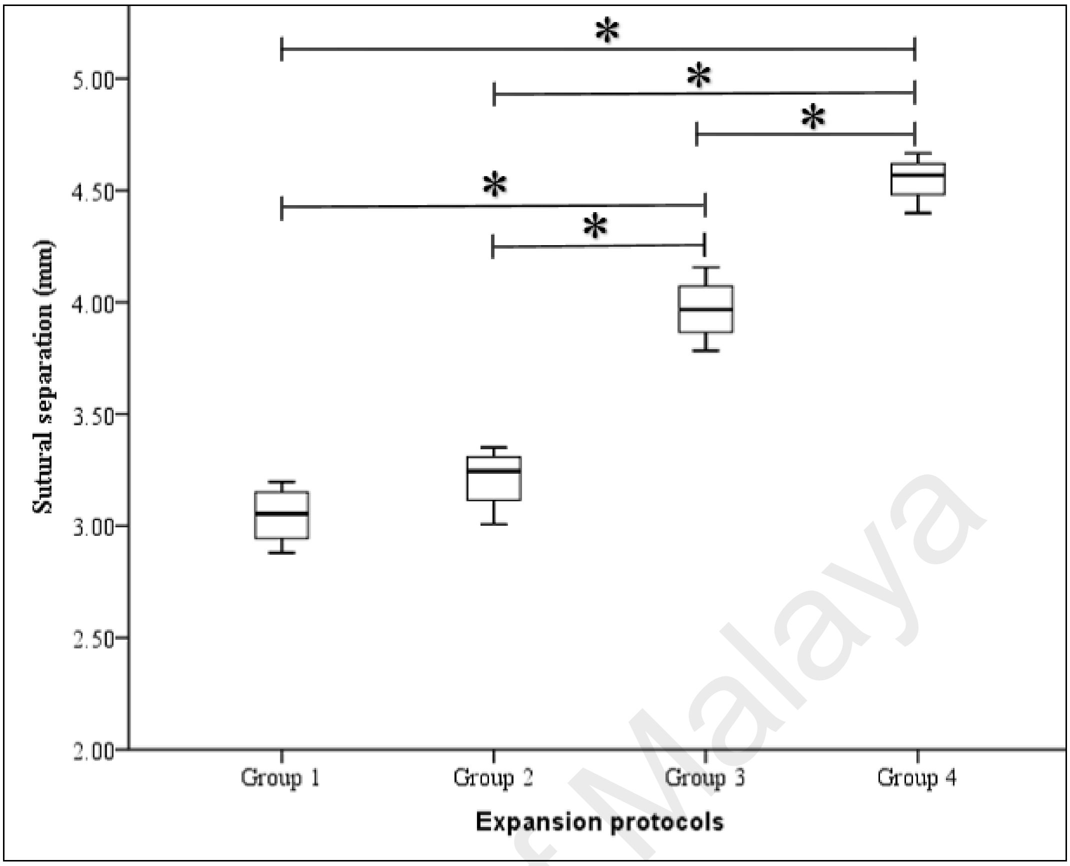


Figure 4.4: Median sutural separation for the various groups of the phase one with the biometric analysis at 6 weeks post retention. Maximum sutural separation was observed in group 4 and the minimum in group 1. * indicates significant differences at p<0.05 (Mann-Whitney U test).

4.3.2 Histomorphometric and immunohistochemical findings

H&E stained sections showed cellularized conjunctive tissue, with young and mature fibroblasts, osteoblast cells, angiogenesis and dense osteoid tissue in the sutural gaps for all experimental groups (Figure 4.5). Bone bridging processes were frequently observed and were formed by bony extensions from the original cortical bone toward the center of the suture gap which were essentially closed in all treatment groups except for group 4. The amount of osteogenic connective tissues within the bony bridge and bone trabeculae in groups 1 and 2 were less than that in the group 3 but were more than group 4. Moreover, the expanded sutures were clearly outlined and filled with a variable quantity of irregular woven bone trabeculae with primitive bone marrow and cellular tissues in groups 1 and 2. Bone trabeculae were observed to be discontinuous in the center of the sutural gaps and continuous at the margins for the later groups. Conversely, thicker bridges and more matured bone trabecula with higher osteoblastic activity and large medullary space that virtually closed the sutural gaps were noted for group 3 (Figures 4.5, 4.6).

Microscopic sections showed dense osteoid tissue, oriented from the margin to the sutural gap center with congested blood vessels, which appeared to be denser in group 3 than in groups 1 and 2. (Figure 4.6). Moreover, areas of bone remodeling and apposition and osteon formation, filled by osteocytes with regular bone disposition, were frequently seen filling the sutural gaps in group 3 (Figure 4.6). In contrast, less bone deposition and remodeling was observed in groups 1 and 2 that virtually disappeared in group 4 (Figure 4.5).

New blood capillaries that were associated with high osteoblastic activity and intense bone deposition and remodeling featured more prominently in group 3. While bony islands and large areas containing bone marrow were observed in the sutural gaps of groups 1 to 3, specimens in group 4 exhibited extensive fibrous tissue consisting of irregular fibroblasts with collagen fibers and less angiogenesis. In addition, initial bone deposition and small, scattered mineralized tissue fragments, surrounded by granulation tissue, were detected near the defect margins in group 4 (Figure 4.6).

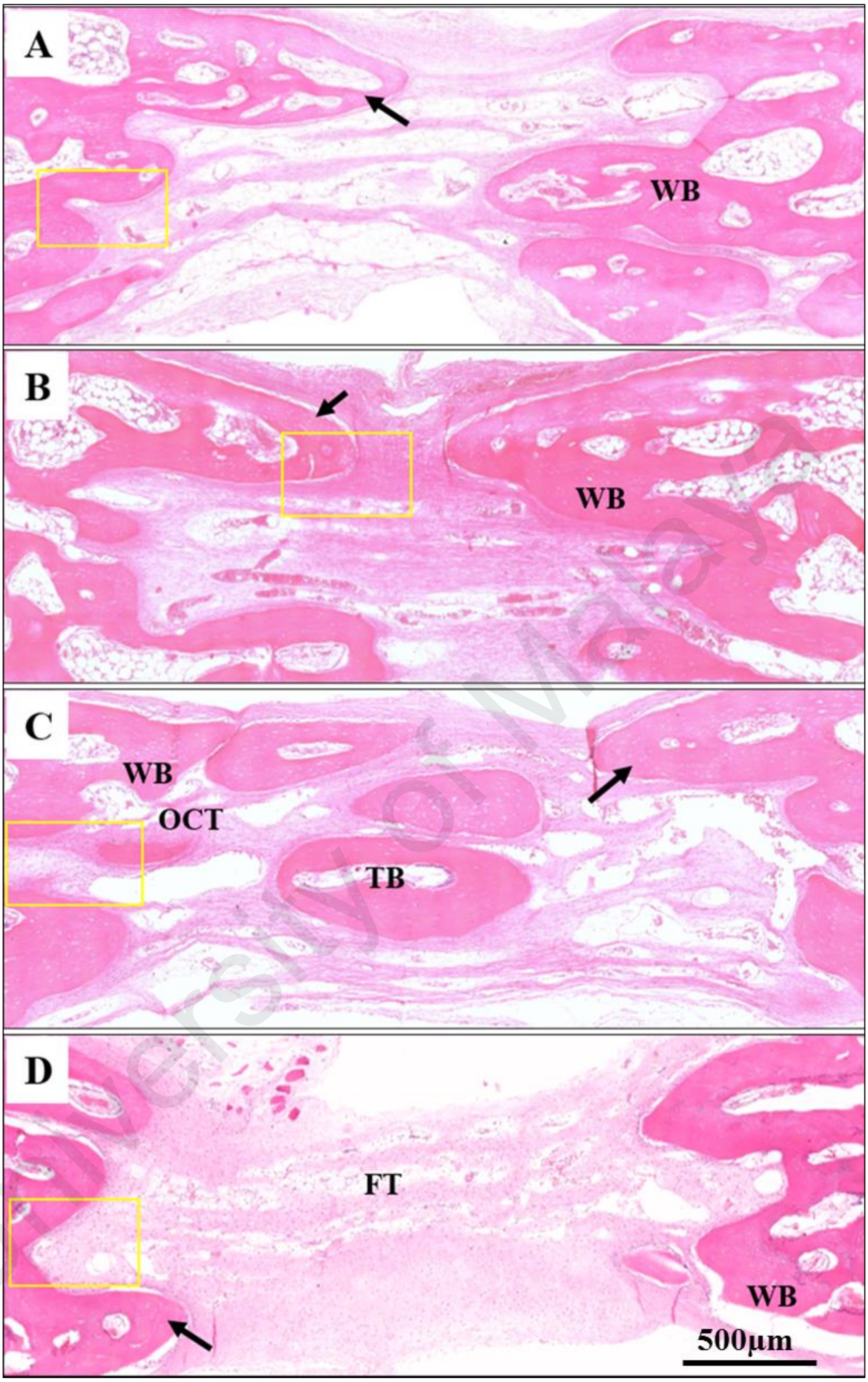


Figure 4.5: Hematoxylin and eosin stained histological sections after 6 weeks of retention at low magnification (4X) (scale bar: 500 µm). The Yellow boxes are presented at a higher magnification in the next figure.

Figure 4.5 showing different lengths of bone projections (black arrows) extending from the margins (native bone) toward the central part of the sutural gaps for all experimental groups (A, B, C, and D for Groups 1, 2, 3, and 4 subsequently). (A & B) Irregular woven bone (WB) with primitive bone marrow and cellular tissues that partially closed the suture; (C) Thicker and more intense bony projections (black arrow) interdigitated with osteoid connective tissue (OCT) almost filled the gap and trabecular bone (TB). (D) Minimal woven bone tissue (WB) that fused to the margin with big fibrous tissue (FT) gap at the center.

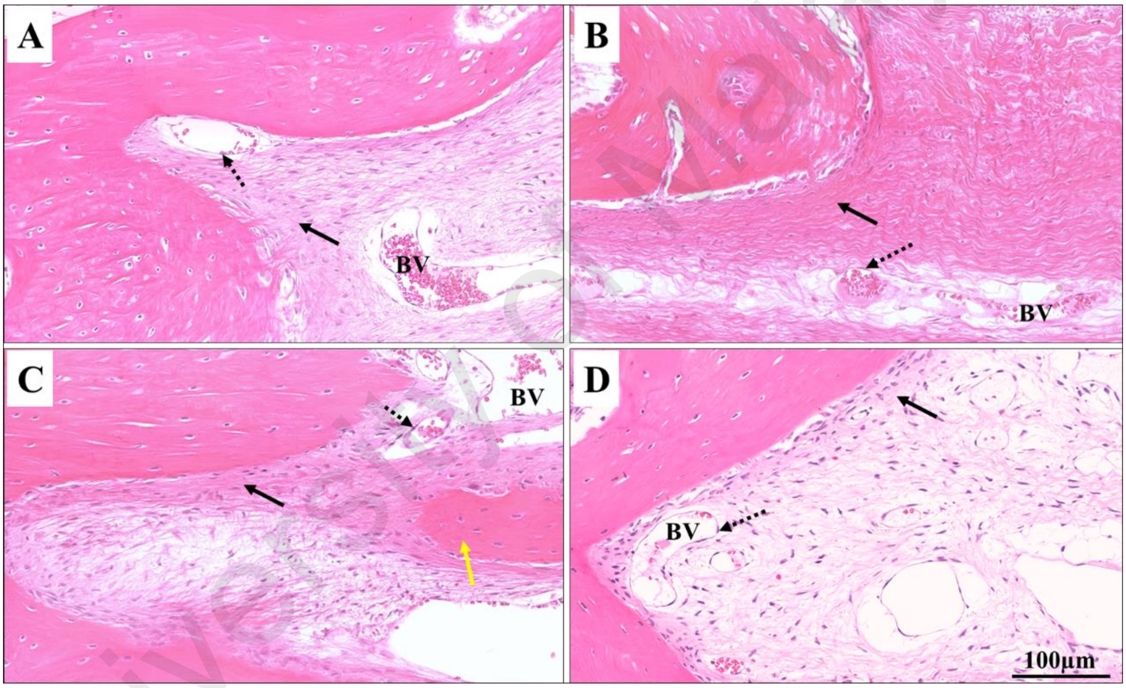


Figure 4.6: Hematoxylin and eosin stained histological sections after 6 weeks of retention at high magnification (20x) (scale bar: 100 µm).

Figure 4.6 showing osteoid tissue that oriented from the margins toward the center of the sutural gap with blood vessels (BV) in all groups (A, B, C, and D for Groups 1, 2, 3, and 4 respectively). Osteoid tissue appeared denser in Group 3 (C) than other groups (black arrows). Osteoblasts lined and deposited osteoid around the blood vessels (doted arrows). Bony Island together with fresh osteoid (yellow arrows) presented in the sutural gap of Group 3 (C).

The results of histomorphometric analysis for the various variables are summarized in Table 4.1. Group 3 showed significantly more (66.93%) while group 4 had significantly less (55.82%) newly formed bone than the other two groups Figure 4.7. There were also significantly less blood capillaries in group 4 than in the other groups. While group 3 had significantly more blood capillaries when compared to group 1 and 4, no statistically significant difference in percentage of blood capillaries was observed between groups 2 and 4. In addition, there was a significantly higher percentage of non-osteoid tissue in group 4 than in the other groups. The percentage of non-osteoid tissue in group 3 was also significantly lower than groups 1 and 4 but comparable to group 2.

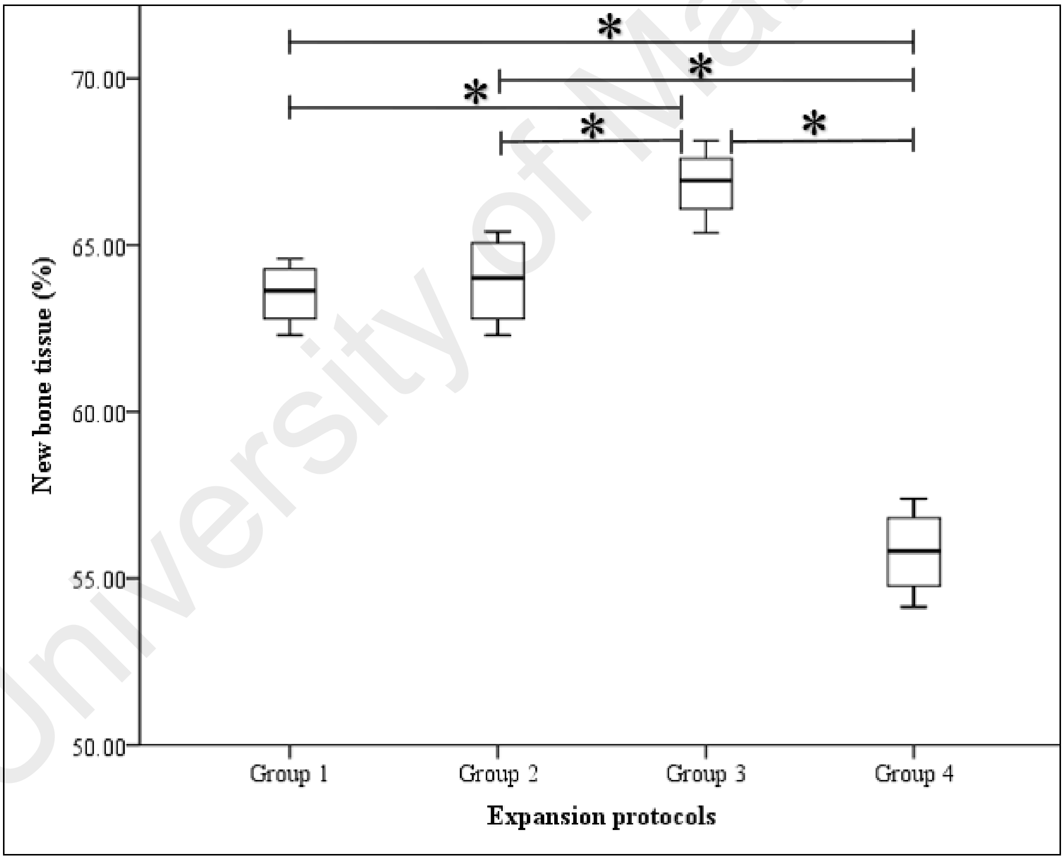


Figure 4.7: Median percentage of new bone formation for the various groups of the phase one with the histomorphometric analysis at 6 weeks post retention. Maximum new bone formation was observed in group 3 and minimum in group 4. * indicates significant differences at p<0.05 (Mann-Whitney U test).

Immunohistochemical sections (Figures 4.8, 4.9) indicated that bone specific proteins (ALP and OPN) were detected extracellularly in the sutural gaps as well as intracellularly in osteoblasts and osteocytes with higher expression near the sutural margins. Morphometric analysis revealed significant differences in median levels of ALP and OPN expression between all 4 experimental groups ($p<0.05$). The highest level of these proteins was attained in group 3, followed be groups 2, 1 and 4 respectively as shown in Table 4.1.

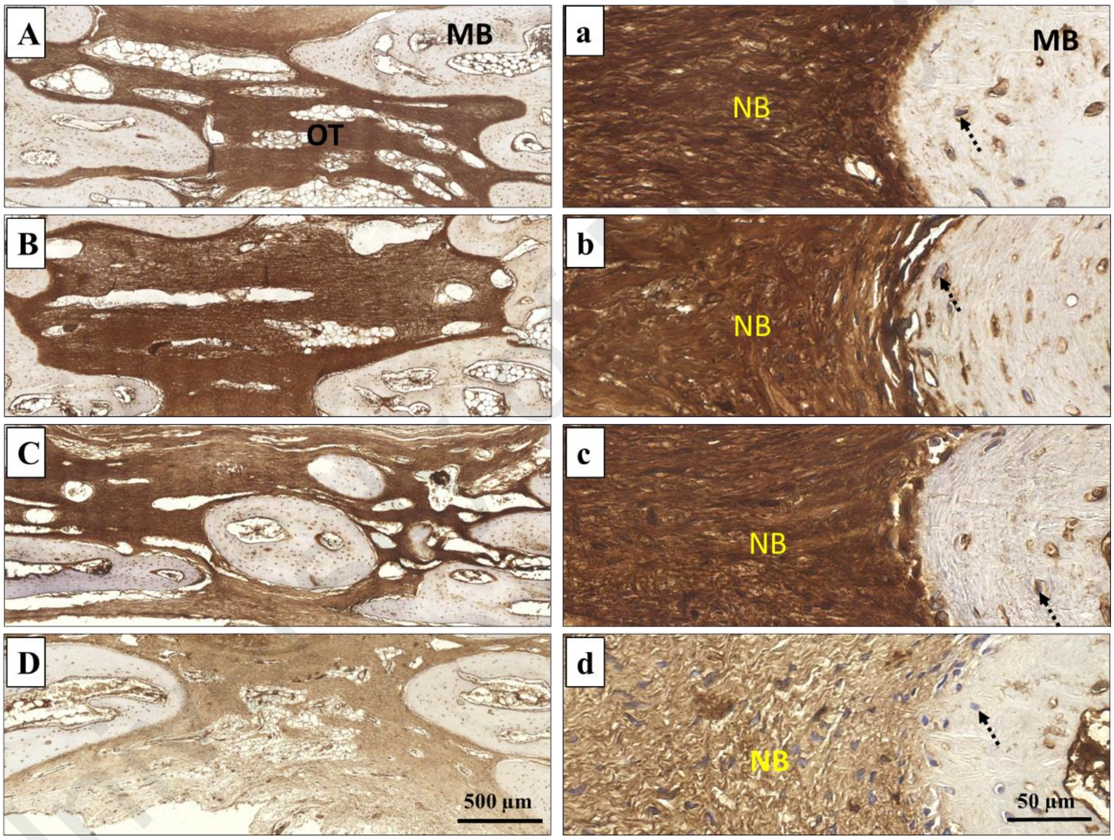


Figure 4.8: Alkaline phosphatase (ALP) immunostaining patterns in the various experimental groups after 6 weeks of retention (A, B, C, and D for Groups 1, 2, 3, and 4 respectively). OT, osteoid tissue; MB, mature bone; Dotted arrow, osteocyte; NB, new bone tissue. The images are arranged in columns by magnification (A, B, C, and D are viewed under 4x; a, b, c, and d are viewed under 40x).

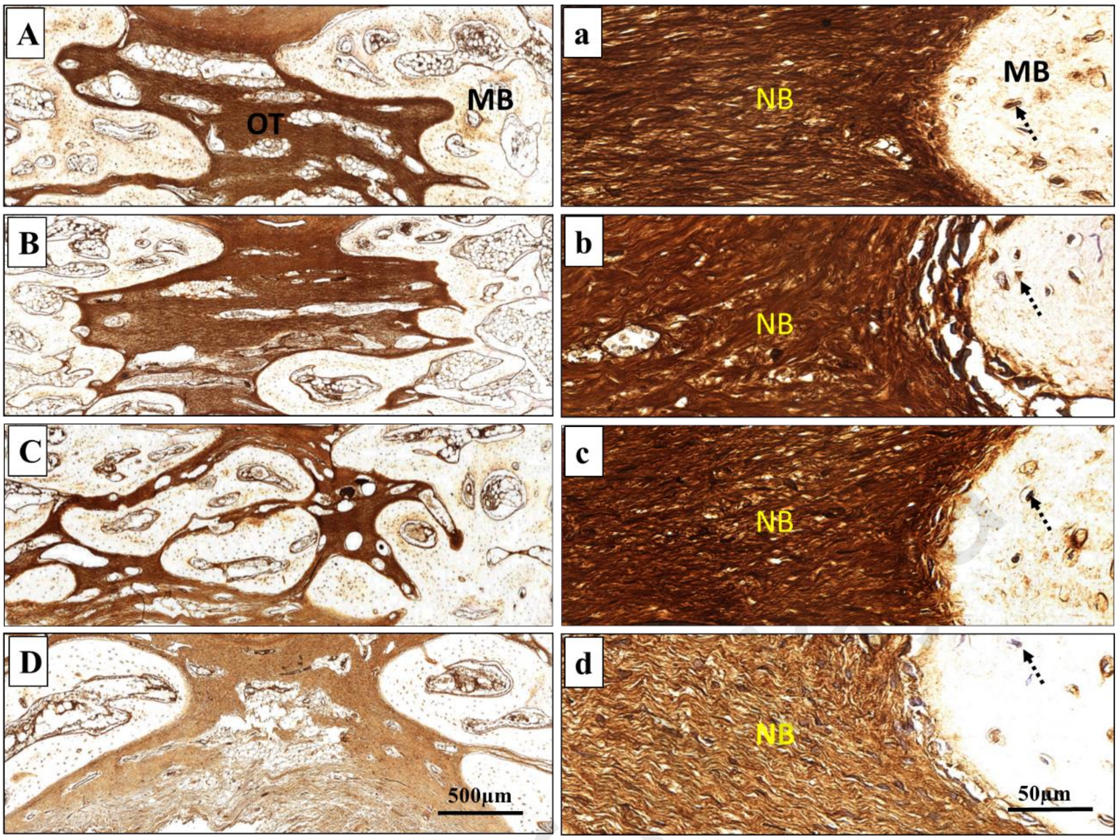


Figure 4.9: Osteopontin (OPN) immunostaining patterns in the various experimental groups after 6 weeks of retention (A, B, C, and D for Groups 1, 2, 3, and 4 respectively). OT, osteoid tissue; MB, mature bone; Dotted arrow, osteocyte; NB, new bone tissue. The images are arranged in columns by magnification (A, B, C, and D are viewed under 4x; a, b, c, and d are viewed under 40x).

Table 4.1: Median sutural separation, bone parameters, histological findings and immunostaining expressions with inter-quartile ranges for the various groups of the phase one at 6 weeks post retention.

Variables			Group 1 (control)	Group 2	Group 3	Group 4	*p* Values
S.S-Micro-CT (mm)	A.S.S	Median	2.99	3.12	3.77	4.55	0.004*
		IQR	2.85-3.04	2.97-3.20	3.71-3.82	4.42-4.63	
	P.S.S	Median	2.71	2.78	3.61	4.27	0.005*
		IQR	2.65-2.78	2.70-2.87	3.53-3.64	4.18-4.35	
	Average	Median	2.84	2.92	3.69	4.41	0.003*
		IQR	2.80- 2.87	2.88- 3.02	3.62- 3.73	4.30- 4.49	
S.S-Biometric (mm)	Average	Median	3.05	3.25	3.97	4.57	
		IQR	2.91-3.17	3.06-3.33	3.82-4.11	4.44-4.64	0.004*
Bone volume fraction BV/TV (%)		Median	66.85	67.14	69.15	59.96	0.005*
		IQR	66.08- 67.66	66.63-67.46	68.54-69.67	58.88-60.45	
Sutural space volume (mm^3)		Median	12.01	10.80	7.57	21.01	0.004*
		IQR	11.40- 12.95	9.70- 12.08	6.86- 8.29	20.40-21.87	
Sutural tissue separation (mm)		Median	0.76	0.73	0.65	1.74	0.004*
		IQR	0.73- 0.79	0.73- 0.74	0.61- 0.68	1.71- 1.80	
New bone formation (%)		Median	63.63	64.01	66.93	55.82	
		IQR	62.54-64.44	62.54-65.24	65.73-67.87	54.46-57.10	0.006*
Blood capillaries (%)		Median	9.41	10.24	10.87	4.48	
		IQR	8.78-10.17	9.72-10.73	10.49-11.13	4.27-5.03	0.006*
Non-osteoid tissue (%)		Median	26.47	24.87	22.71	39.62	
		IQR	26.01-27.44	24.04-26.37	21.7824.30	38.17-41.28	0.006*
ALP (%)		Median	62.21	62.87	68.00	49.62	
		IQR	57.20-66.29	58.81-66.89	65.90-72.69	45.63-52.39	0.010*
OPN (%)		Median	57.48	61.14	64.11	43.72	
		IQR	53.13-62.25	58.65-64.74	62.89-67.75	41.30-46.94	0.012*

*indicates statistically significant differences among the different groups. Results of Kruskal-Wallis test ($p<0.05$). A.S.S, anterior sutural separation; P.S.S, posterior sutural separation; S.S-Micro-CT, sutural separation measured with micro-CT; S.S-Biometric, sutural separation measured with biometric analysis; ALP, alkaline phosphatase antibody; OPN, osteopontin antibody.

4.4 Micro-computed tomography analysis for the phase two

4.4.1 Sutural separation

Median values for average sutural separations were 3.69, 5.52 and 4.97 mm for groups 1 to 3, respectively (Table 4.2). Paired comparisons showed statistically significant differences in sutural separation between all groups (Figure 4.10). Sutural separation in the corticotomized animals (groups 2 and 3) were significantly greater than group without corticotomy (group 1). Continuous corticotomy (group 2) yielded larger sutural separation, then discontinuous corticotomy. Sutural separation for groups 2 and 3 were in fact 49.59% and 34.69% greater than group 1, respectively.

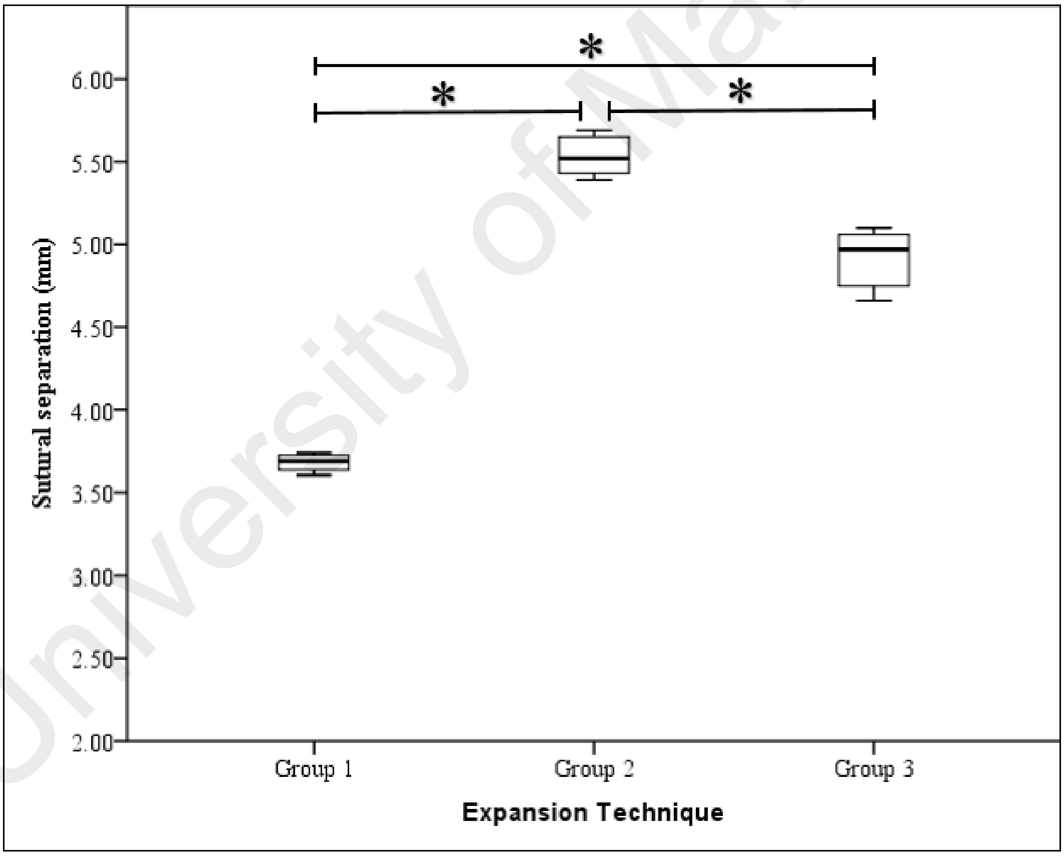


Figure 4.10: Median sutural separation (mm) for the various groups of the phase two with micro-CT at 6 weeks post retention. Maximum sutural separation was observed in group 2 (5.52 mm) and the minimum in group 1 (3.69 mm). * indicates significant differences at p<0.05 (Mann-Whitney U test).

4.4.2 Bone parameters

Figure 4.11 shows the 2D images for ROI, 3D color map and 3D segmentation for the various groups of the phase two at 6 weeks post retention. From 3D color map, we noted that the amount of suture tissue separation (red color) was almost the same in groups 2 and 3 (corticotomized groups) and maximum in group 1. Furthermore, the expanded sutures were filled with a new bone formation that completely closed the sutural gap in group 2, while in group 1 was virtually closed the sutural gap.

Median BV/TV, sutural space volume, and sutural tissue separation after 6 weeks of retention are also reflected in Table 4.2. Group 2 had significantly higher BV/TV than the other two groups. Group 3 had non-significant higher BV/TV than group 1 (control) (Figure 4.12). The amount of sutural space volume and sutural tissue separation in group 2 were significantly lower than the other groups, while in group 1, it was non-significantly higher than group 3. Spearman's correlation showed strong, positive and significant association (r= 0.906, $p<0.01$) between BV/TV and sutural separation. In contrast, the correlation between BV/TV and sutural space volume was strong, negative and significant (r= -0.953, $p<0.01$).

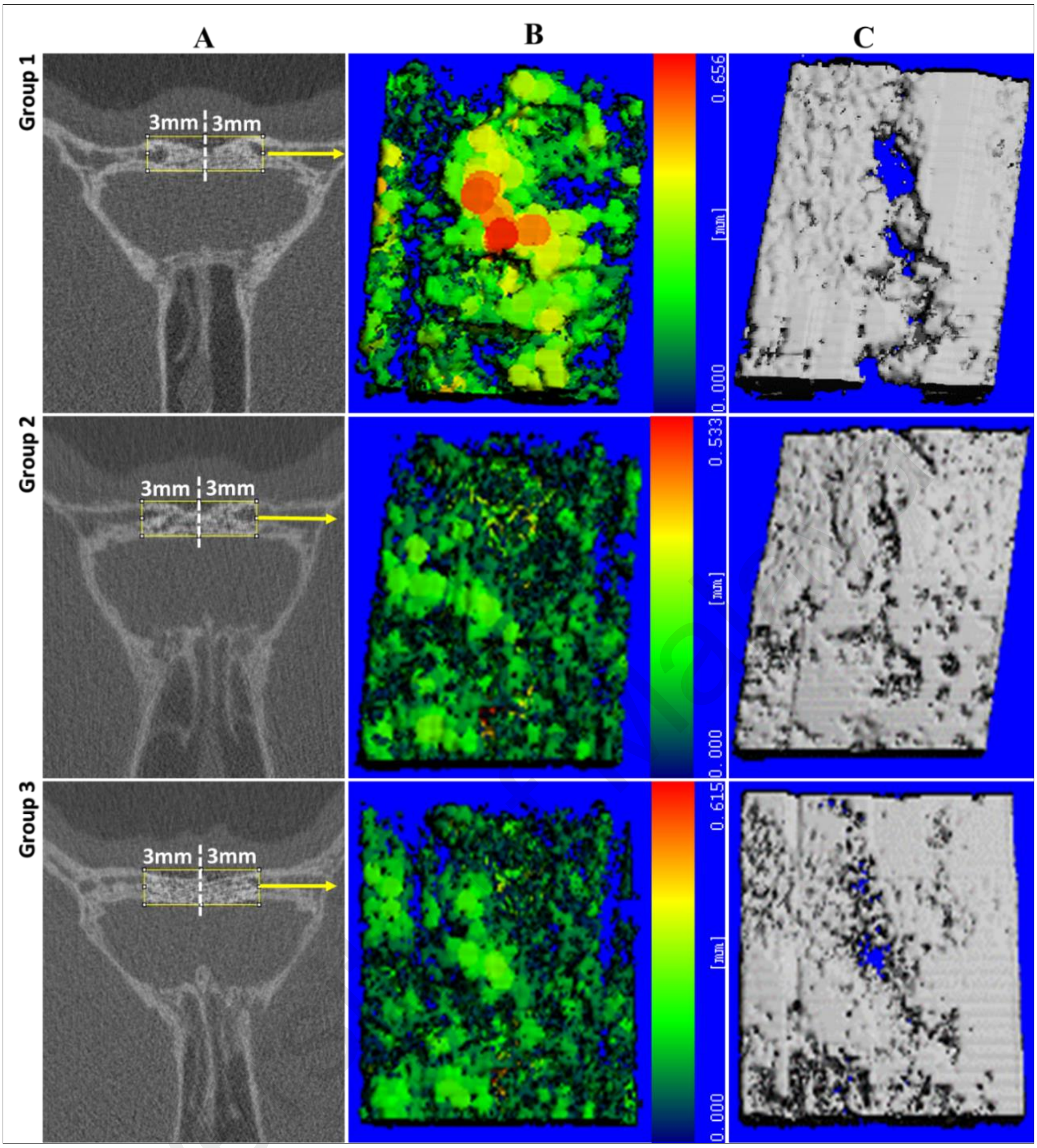


Figure 4.11: (A) 2D image showing the regions of interest for the various groups of the phase two at 6 weeks post retention, (B) 3D map showing the degree of suture tissue separation (blue indicates no tissue separation while red indicates maximum separation), (C) 3D segmentation showing the sutural space. The white dotted line (A) represented the midline of the suture. ROI was 3 mm from the right and left side of the suture.

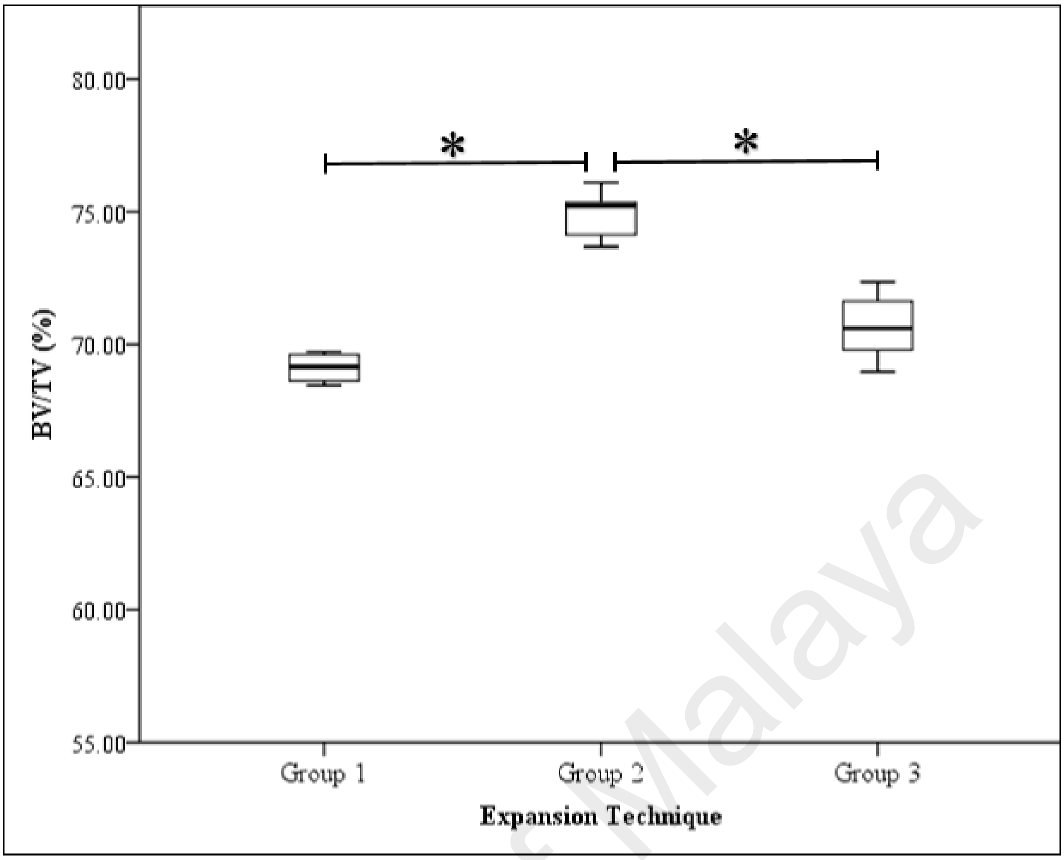


Figure 4.12: Median bone volume fraction changes % (BV/TV) for the various groups of the phase two with micro-CT. Maximum bone volume fraction was observed in group 2 (continuous corticotomy) and minimum in group 1 (without corticotomy). * indicates significant differences at p<0.05 (Mann-Whitney U test).

4.5 Histomorphometric and immunohistochemical analysis for the phase two

4.5.1 Sutural separation

Biometric analysis of histological sections showed median sutural separations of 3.97, 5.58 and 4.88 mm for groups 1 to 3, respectively (Table 4.2). Paired comparisons showed statistically significant differences in biometric sutural expansion between groups (Figure 4.13). Groups 2 and 3 (corticotomized groups) had significantly greater sutural separation than group 1 (not corticotomized group). Sutural separation for group 2 (continuous) was significantly larger than group 3 (discontinuous). Sutural separation for groups 2 and 3 were in fact 40.55% and 22.92% greater than group 1, respectively.

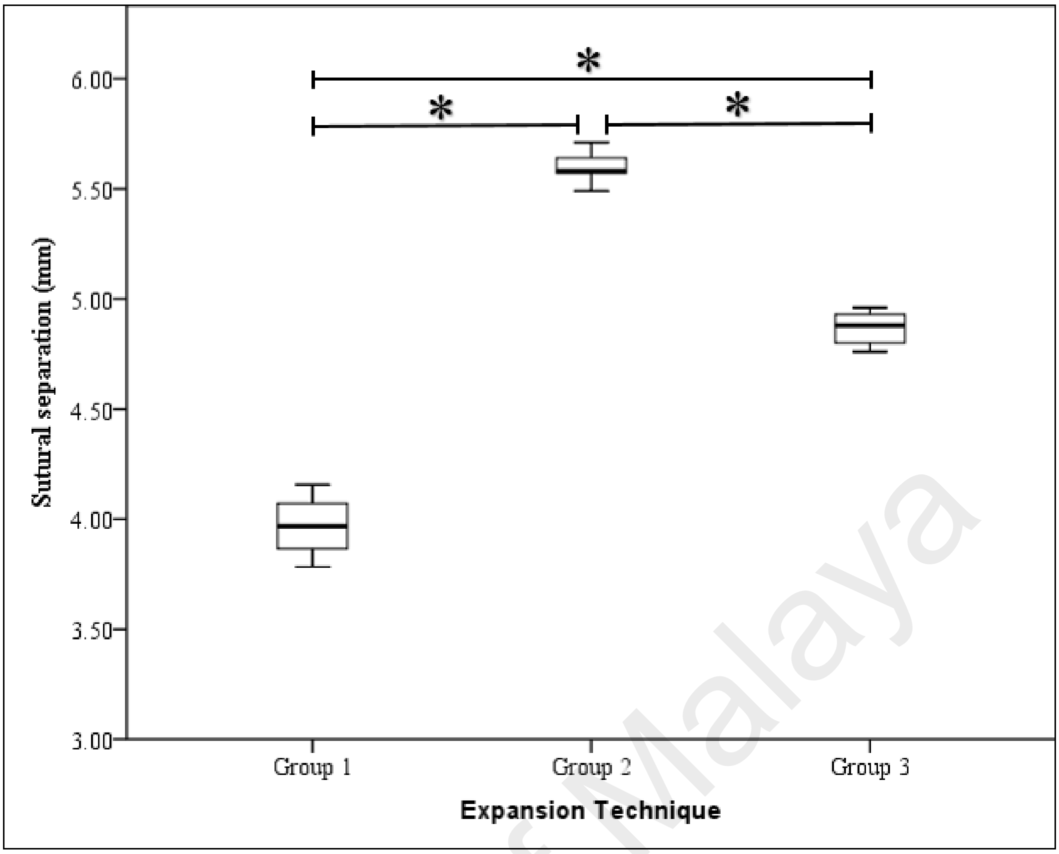


Figure 4.13: Median sutural separation (mm) for the various groups of the phase two with biometric analysis at 6 weeks post retention. Maximum sutural separation was observed in group 2 (5.58 mm) and the minimum in group 1 (3.97 mm). * indicates significant differences at p<0.05 (Mann-Whitney U test).

4.5.2 Histomorphometric and immunohistochemical findings

H&E stained sections for group 1 (control) showed thick bridges and matured bone trabecula with osteoblastic activity and medullary space that virtually closed the sutural gaps. In addition, dense osteoid tissue, oriented from the margin to the sutural gap center with congested blood vessels was observed (Figure 4.14). Furthermore, areas of bone remodeling and apposition and osteon formation, filled by osteocytes with regular bone disposition, were frequently seen filling the sutural gaps in this group (Figure 4.15). Bony islands and areas containing bone marrow that observed in the sutural gaps of group 1 were less obvious than group 2 (continuous sutural corticotomy).

In continuous sutural piezoelectric corticotomy (group 2), a microphotograph of the histological section demonstrated regenerate bone between distracted segments, more areas of new bone formation (Figure 4.14) lined by osteoblast cells, with the presence of numerous blood vessels, newly formed osteocytes, as well as intense osteogenic activity (Figure 4.15). This tissue was characterized by a presence of trabecular tissue with connective tissue on the interim (osteoid tissue) and mature bone tissue on the margin of the defect (Figure 4.14). The distraction gap was found to be filled with the mature and immature bone with more angiogenesis that was associated with high osteoblastic activity and intense bone deposition. New blood capillaries that were associated with high osteoblastic activity and intense bone deposition and remodeling featured more prominently in this group than group 1 (Figure 4.15).

The histologic section of group 3 (discontinuous corticotomy) demonstrated less bone formation than the continuous one but more than group 1 (control) as shown in Figure 4.14. It also exhibited less trabeculae and more fibrous callus occupying most areas of the expanded suture with more osteoclastic cells (Figure 4.15). showed osteoid tissue that were oriented from the margins toward the center of the sutural gap with congested blood vessels (BV) in all groups (A, B, and C, for groups 1, 2, and 3, respectively).

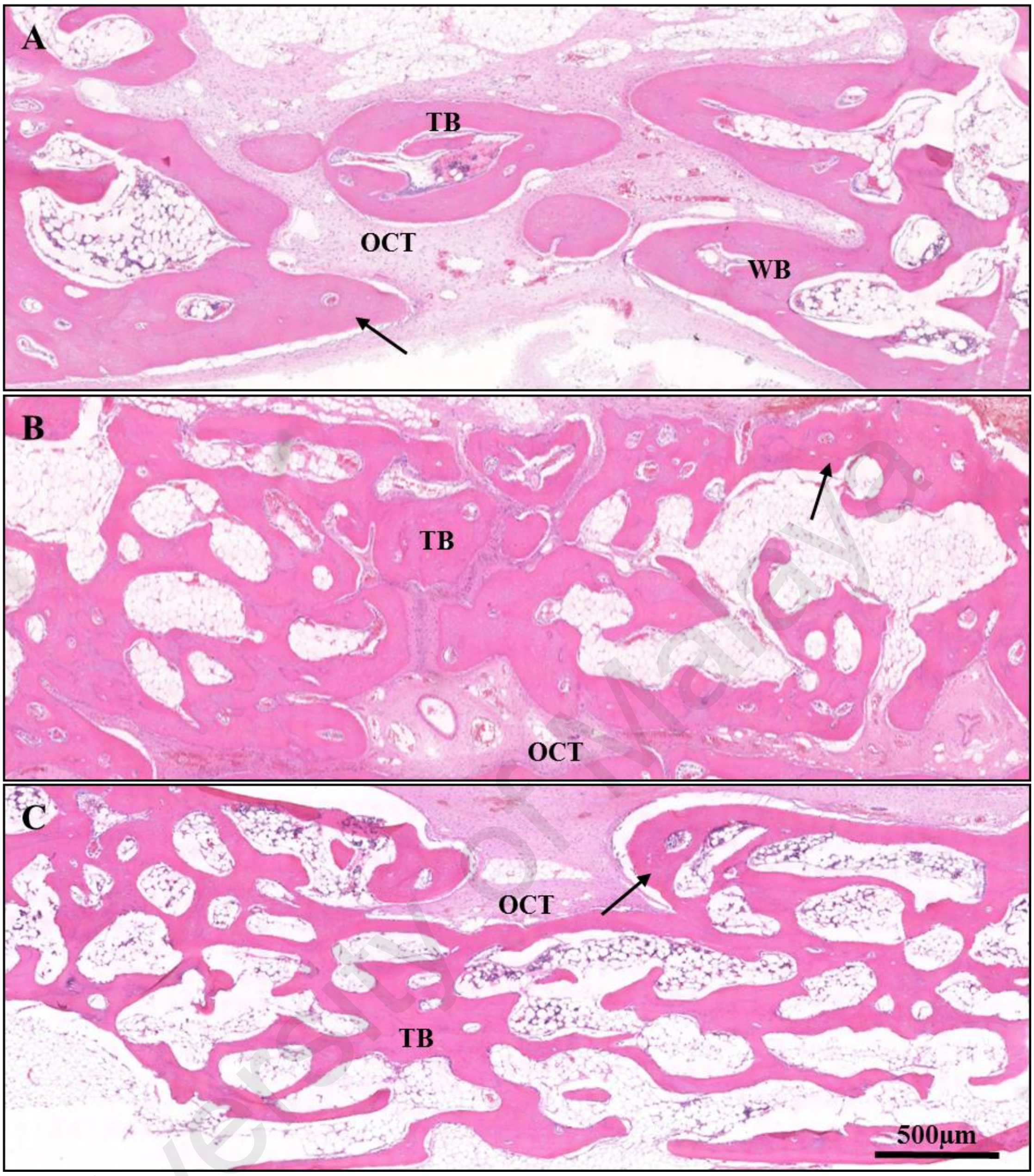


Figure 4.14: Hematoxylin and eosin stained histological sections at 4X magnification for the various groups of the phase two at 6 weeks post retention (A, B, and C for Groups 1, 2, and 3 respectively) (scale bar: 500 µm).

Figure 4.14 showing different lengths of bone projections (black arrows) extending from the margins (native bone) toward the central part of the sutural gaps for all groups (A, B, and C, for groups 1, 2, and 3, subsequently). (A) irregular woven bone (WB) with primitive bone marrow, island trabecular bone (TB) and cellular tissues that partially closed the suture; (B) thicker and longer bony projections (black arrow) inter-digitated with osteoid connective tissue (OCT) filled the gap and trabecular bone (TB).

(C) Thick and intense bony projections (black arrow) interdigitated with osteoid connective tissue (OCT) filled the gap and trabecular bone (TB).

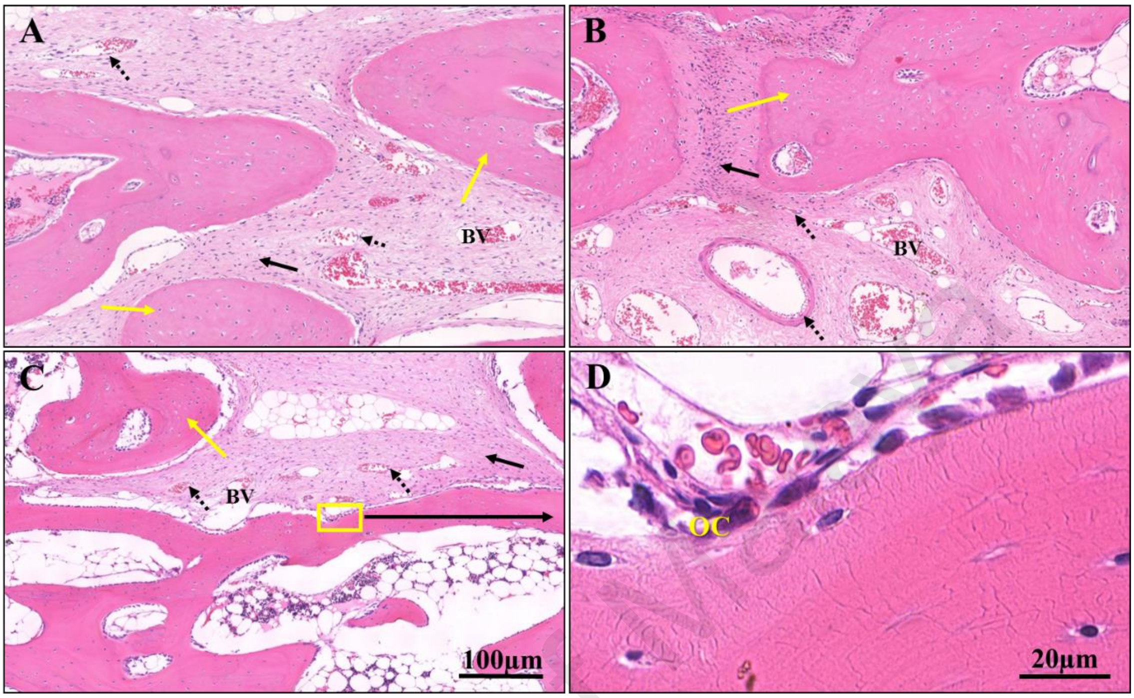


Figure 4.15: Hematoxylin and eosin stained histological sections at 20X magnification for the various groups of the phase two at 6 weeks post retention (A, B, and C for Groups 1, 2, and 3 respectively).

Figure 4.15 showing osteoid tissue which is appeared denser in group 2 (B) than other groups (black arrows) with more blood vessels (BV). Osteoblasts lined and deposited osteoid around the blood vessels (dotted arrows). Bony Island together with fresh osteoid (yellow arrows) presented in the sutural gap of all groups. D) Histological section represents the yellow box at high magnification (100X) to show osteoclasts cell (OC).

Statistically, group 2 showed significantly more new bone formation (76.25%) than the other two groups, followed by groups 3 and 1 (Figure 4.16). A significantly higher blood capillaries were observed in group 2 when compared to the other groups, while group 3 had significantly more blood capillaries when compared to group 1. In addition, the proportion of non-osteoid tissue in group 1 was significantly higher than in the other groups, while in group 2 it was significantly lower than groups 3.

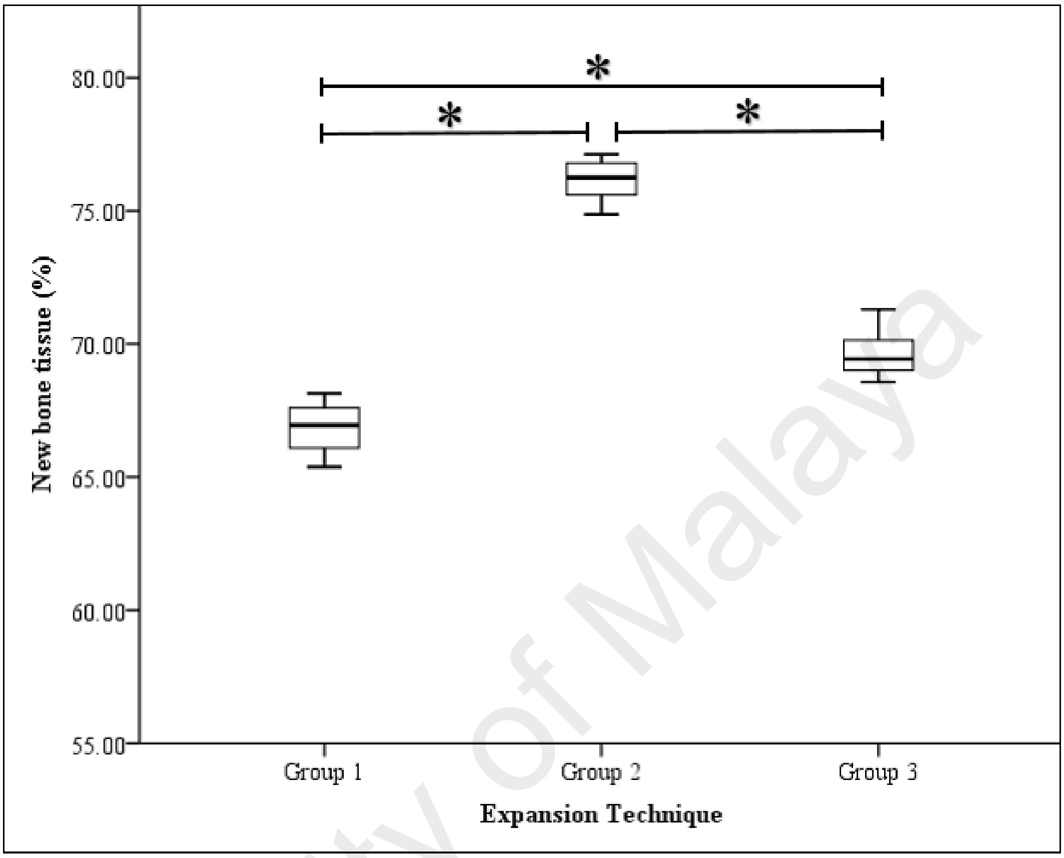


Figure 4.16: Median percentage of new bone formation for the various groups of the phase two with histomorphometric analysis at 6 weeks post retention. Maximum new bone formation was observed in group 2 (continuous corticotomy) and minimum in group 1 (without corticotomy). * indicates significant differences at p<0.05 (Mann-Whitney U test).

In regards to immunohistochemical analysis (Figures 4.17, 4.18), group 2 showed significantly higher ALP expression than other groups. Group 3 had non-significantly higher expression of ALP in comparison to group 1. The differences in OPN expression were non-significant between the various groups. The highest amount was observed in group 2 followed by groups 3 and 1, respectively. Spearman's correlation test showed strong, positive and significant correlation ($r = 0.881$, $p<0.01$) between new sutural bone formation and the amount of sutural separation.

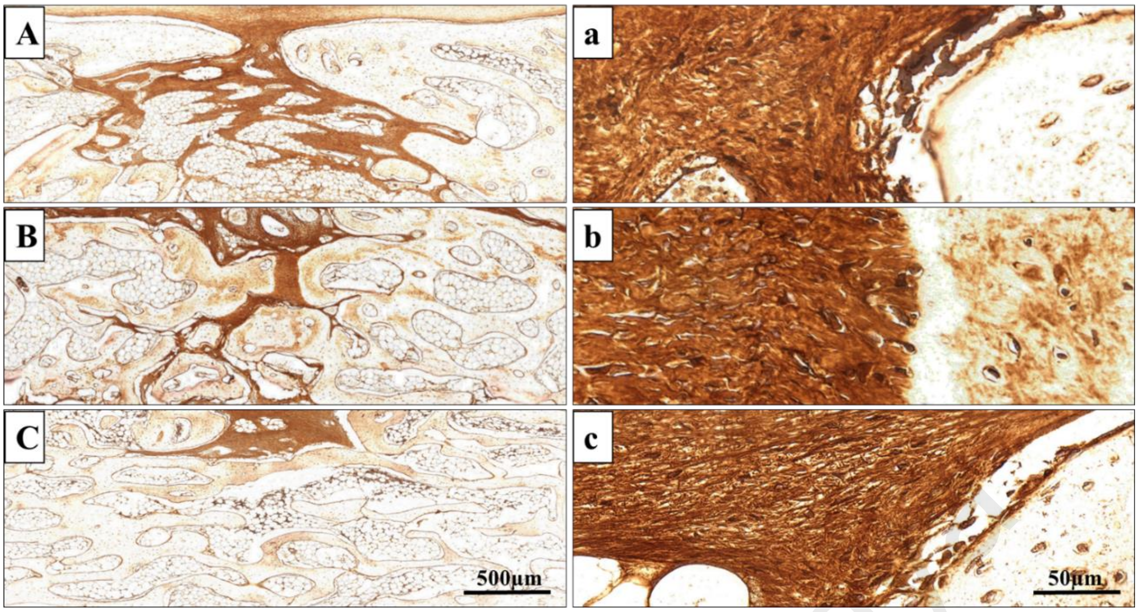


Figure 4.17: Alkaline phosphatase (ALP) immunostaining patterns in the various groups for the phase two at 6 weeks post retention (A, B, and C for Groups 1, 2, and 3 respectively). The images are arranged in columns by magnification (A, B, and C are viewed under 4X; a, b, and c are viewed under 40X).

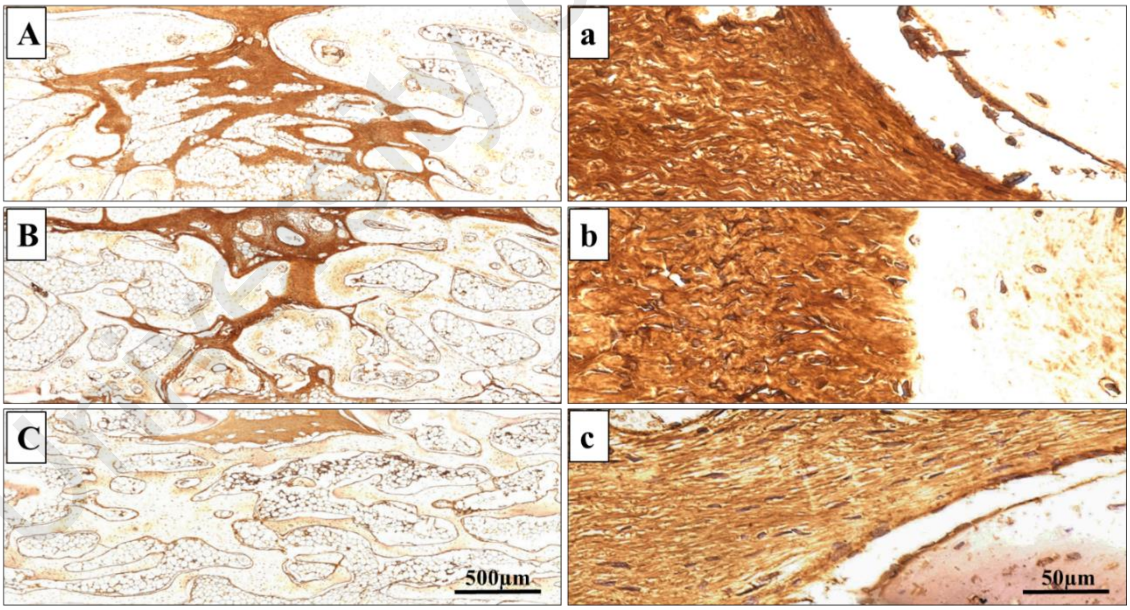


Figure 4.18: Osteopontin (OPN) immunostaining patterns in the various groups for the phase two at 6 weeks post retention (A, B, and C for Groups 1, 2, and 3 respectively). The images are arranged in columns by magnification (A, B, and C are viewed under 4X; a, b, and c are viewed under 40X).

Table 4.2: Median sutural separation, bone parameters, histological findings and immunostaining expressions with inter-quartile ranges for the various groups of the phase two at 6 weeks post retention.

Variables		Group 1 (control)	Group 2	Group 3	*p* Values
Sutural separation (mm) Micro-CT	Median	3.69	5.52	4.97	0.003*
	IQR	3.62- 3.73	5.41-5.67	4.71-5.10	
Sutural separation (mm) Biometric	Median	3.97	5.58	4.88	
	IQR	3.82-4.11	5.53-5.68	4.78-4.95	0.003*
Bone volume fraction BV/TV (%)	Median	69.15	75.22	70.60	0.005*
	IQR	68.54- 69.67	73.91- 75.73	69.38- 71.99	
Sutural space volume (mm^3)	Median	7.57	3.46	7.22	0.010
	IQR	6.86-8.29	3.31-4.13	6.79-7.92	
Sutural tissue separation (mm)	Median	0.65	0.51	0.62	0.010*
	IQR	0.61- 0.68	0.45- 0.52	0.61- 0.66	
New bone formation (%)	Median	66.93	76.25	69.43	
	IQR	65.73-67.87	75.24-76.96	68.78-70.72	0.003*
Blood capillaries (%)	Median	10.87	16.65	11.47	
	IQR	10.49-11.13	16.03-17.31	11.13-12.74	0.004*
Non-osteoid tissue (%)	Median	22.71	6.58	19.10	
	IQR	21.78-24.30	6.00-8.74	16.55-20.10	0.003*
ALP (%)	Median	68.00	76.45	70.30	0.020*
	IQR	65.90-72.69	75.10-79.25	68.00-73.73	
OPN (%)	Median	64.11	69.10	66.54	0.158
	IQR	62.89-67.75	65.24-71.95	64.62-68.06	

*indicates statistically significant differences among the different groups. Results of Kruskal-Wallis test ($p<0.05$). ALP, alkaline phosphatase antibody; OPN, osteopontin antibody.

CHAPTER 5: DISCUSSION

5.1 Animal selection and preparation

The midsagittal suture of the rabbits had been proposed as an animal model for palatal expansion (Pulver et al., 2016) and was selected for our study. The midsagittal sutures of rabbits were found to act analogously to the midpalatal sutures. In addition, the Haversian systems of rabbits were found to be similar to humans, which allowed for some extrapolation of results to clinical applications (Liu et al., 2011). Furthermore, the rabbits were adequately small for use with micro-CT, permitting whole body insertion into the micro-CT chamber for repeated measurements. The frontal areas were also sufficiently wide for the fixation of the palatal expander and 4 miniscrew implants. In terms of age, adult rabbit was chosen to reduce confounding factors arising from continuous growth on bone remodeling (Suckow & Douglas, 1997). With regards to gender, male rabbits were selected to reduce the effects of hormonal variations and to capitalize on the wide frontal bone of male rabbits.

5.2 Phase one: Optimal instant expansion

Approximately 8% to 18% of patients with mixed-dentition have transverse maxillary constrictions (Dasilva et al., 1991) and are frequently managed with tooth-borne RME appliances. Although tooth-borne RME appliances vary in their designs and rate of expansion (Bench, 1998; Mundstock et al., 2007), their fundamental mechanisms remain the same (Proffit et al., 2014). Rapid transverse forces are exerted on the maxillary teeth resulting in interruption and separation of the midpalatal sutures. The latter leads to extensive cellular activity in the sutures and encourages bone remodeling (Starnbach et al., 1966). As tooth-borne RME appliances transmit expansion forces through teeth, dental and alveolar bone bending also occurs during the correction of the skeletal disharmonies (Graber et al., 2011). These movements take up the major fraction of total appliance activation, reducing the amount of true skeletal expansion (Basciftci & Karaman, 2002; Garrett et al., 2008).

An alternative approach is to apply expansion forces directly to the midpalatal suture with bone-borne RME. While ankylosed teeth and aluminum oxide implants were used in animal models (Guyman et al., 1980; Turley et al., 1980), osteosynthesis plates (Gerlach & Zahl, 2003; Klier et al., 2005) were employed for transferring expansion forces directly to bone in patients. The latter had several disadvantages, including the need for invasive surgical procedures with higher risk of infections (Klier et al., 2005). Speech problems may also arise due to limitation of tongue movements caused by the relatively large size of the plates and expanders. More recently, miniscrew implant assisted bone-borne RME appliances were introduced (Carlson et al., 2016; Lagravère et al., 2010; Proffit et al., 2014). These bone-borne RME appliances produced greater orthopedic effects and fewer dentoalveolar side effects when compared to tooth-borne ones (Lin et al., 2014). Furthermore, the major advantage of bone-borne RME over tooth-borne RME is the fact that the expansion forces are acting directly to the bone at the mechanically desired level. This avoids anchorage-tooth tipping, excludes orthodontic relapse, and keeps segmental maxillary tipping to a minimum, leading to less skeletal relapse (Matteini & Mommaerts, 2001; Mommaerts, 1999; Northway & Meade Jr, 1997). Moreover, they are well-tolerated and easier to use than traditional tooth-borne expanders (Garreau et al., 2016).

The key objective of bone-borne RME is the expansion of the midpalatal suture. Despite its extensive clinical and research history, the optimal force and instant expansion that can be achieved without compromising sutural bone formation has not been established. The phase one of the present study investigated the effects of accelerated bone-borne expansion protocols on sutural separation and sutural bone formation using micro-CT analysis, histomorphometry/immunohistochemistry, and determined the optimum instant sutural expansion possible without affecting the ability of suture to bone remodel. A significant difference in sutural separation, bone volume fraction, new bone formation and quantity of immunohistochemical markers were observed between the various expansion protocols and a critical amount of instant expansion was existed.

Anchorage for the customized expanders was achieved with miniscrew implants. The success rate of the miniscrew implants (98.44%) was relatively higher than that reported by Liu and coworkers (88%) based on a similar rabbit model. The incongruity may be attributed to the use of more and longer miniscrew implants in our study (5mm versus 3mm) and the strength of the laser welded joint between the 'U' loops and Hyrax expanders. Moreover, Liu and coworkers used nickel-titanium open-coil springs that provided limited control over the direction and amount of forces placed on the miniscrew implants (Liu et al., 2011). Our success rate was similar to that of Carrillo and colleagues who reported 99% success when miniscrew implants were used for orthodontic anchorage in beagle dogs (Carrillo et al., 2007). Garfinkle and coworkers found that loaded miniscrew implants have a higher success rate than unloaded ones and proposed that applied forces augment initial mechanical retention and stimulate osseous adaptation (Garfinkle et al., 2008). This was corroborated by the high success rate of the loaded miniscrew implants in our study.

For all groups, the amount of sutural separation was slightly greater anteriorly than posteriorly (Table 4.1). This observation was consistent with those reported by Liu and coworkers (Liu et al., 2011). The difference between the anterior and posterior segments was, however, statistically insignificant. The pattern of differential sutural expansion had also been reported in humans as the posterior part of the midpalatal suture articulates with more bones than the anterior portion (Akin et al., 2015; Wertz, 1970).

The frontal bone in rabbits articulates with the nasal bone anteriorly and parietal bone posteriorly. As the parietal bone connects to more skeletal structures than the nasal bone, more skeletal resistance and less sutural expansion is anticipated posteriorly.

In the present study, the expanded suture was irregular in shape with the inner surface (toward the brain) less enlarged than the outer surface (toward mucosal). These irregularities may be attributed to differential bone remodeling between the inner and outer surface of the sutures. Generally, during surgical incisions, the periosteum is detached from the outer surface of the midsagittal suture and adjacent bone. This could lead to disruption of the periosteal microcirculation to the suture and osteogenic potential of the periosteum. As a result, periosteal blood flow reduction and subsequently decreases bone remodeling occurs. In contrast, the inner surface of the suture and adjacent bone is still covered by periosteum, resulting in greater bone remodeling (Squier et al., 1990; Stoetzer et al., 2014). Sutural separation escalated with increasing instant expansion (Figure 4.1). A higher instant sutural expansion might have loosened the midsagittal suture reducing bony resistance and allowing for more sutural separation.

In distraction osteogenesis, increased amount of new bone formation appeared to result from rapid recruitment and activation of bone-forming cells and the increased surface area available for matrix deposition and mineralization (Welch et al., 1998). Although bone volume fraction responded to increasing instant expansion, a critical point was reached with an instant expansion of 2.5 mm (Figure 4.3). Bone volume fraction conversely decreased about 13.29% when instant expansion was increased to 4 mm. As instant expansion increases, expansion forces will escalate correspondingly. The forces exerted at 4 mm instant expansion are probably too extreme leading to the decreased bone volume fraction observed (Figure 4.3). DNA synthesis of fibroblasts and osteoblasts plateaus after a certain expansion force magnitude. Excessive forces inhibit anabolic activities due to tissue rupture as well as bleeding (Mörndal, 1987) and can result in cell death (Zahrowski&Turley, 1992). These events might explain the negative correlation between bone volume fraction and amount of sutural space volume observed in our study.

The rate of distraction (incremental lengthening is performed per day) had also been identified as a significant factor in the new sutural bone formation and consolidation (Ilizarov et al., 1969; Leong et al., 1979). Higher rates of distraction also enhanced bone formation, but only to a critical measure. Ilizarov and coworkers reported that a rate of 60 times per day resulted in a significantly greater new bone formation when compared with lower rates of 1 to 4 times per day (Ilizarov et al., 1969). The rate of gradual distraction during the active phase was fixed at 2 turns (0.5 mm) per day in the present study. The collective effect of increased instant suture expansion and rates of gradual distraction warrants further investigation.

Findings from histomorphometric evaluation had been steady with those from micro-CT analysis. According to the histomorphometric investigation, the extent of sutural separation in groups 3 and 4 were 30.16 and 49.84% greater than the control group (group 1). The amount of new bone formation was, however, higher in group 3 than that in other experimental groups. With reference to group 1 (control), new bone formation was 5.19% greater and 12.27% lesser in groups 3 and 4, respectively (Figure 4.7). This inferred that the relationship between sutural separation and new bone formation was generally linear up to a critical limit beyond which new bone formation was disrupted. In the present study, the critical sutural separation was found to be about 3.97 mm that accompanied group 3. During expansion, the collagen fibers that connect the suture edges are stretched together with periosteum surrounding the bony margins, leading to the initiation of sutural bone formation (Murray & Cleall, 1971). Larger instant expansions are anticipated to induce greater stretching of the periosteum and collagen fibers resulting in more bone formation (Parr et al., 1997). When stretched at once beyond 2.5 mm, new bone formation reduced considerably and may be attributed in part to collagen fiber and other tissue damage arising from their over-extension.

The larger amount of new bone formation in group 3 was coupled with a higher percentage of blood capillaries. The larger distraction may provide a better blood supply to the sutural site (Mizuta et al., 2003). Local blood supply affects the pattern of bone-forming process with bone formation occurring in regions with adequate vascularity, and cartilage formation in ischemic areas (Carter et al., 1998). The pericytes neighboring the microvasculature are stimulated when new vessels are formed and migrate along the newly formed capillaries. These pericytes are deemed to be a source of osteoblasts in an orthopedically expanded suture. They appear to play a significant role in angiogenesis and consequent osteogenesis (Chang et al., 1997) and accounts for the increased new bone formation with increased blood capillaries.

As highlighted earlier, the amount of new bone formation decreased when instant expansion was increased from 2.5 (group 3) to 4 mm (group 4). The latter resulted in a total median sutural separation of 4.57 mm. When instant expansion increases, expansion forces will escalate correspondingly. The force exerted from 4 mm instant expansion are probably too extreme leading to decreased blood capillaries as well as new bone formation. As mentioned before, DNA synthesis of fibroblasts and osteoblasts was reported to plateau after a certain expansion force magnitude. Excessive forces inhibit anabolic activities due to tissue rupture as well as bleeding (Mörndal, 1987) leading to cell death (Zahrowski & Turley, 1992).

Outcomes from immunohistochemical analysis were consistent with those from histomorphometric investigations. ALP is a bone specific-protein that is commonly used as a marker for osteoblast cells. It plays an important role in the interaction between bone cells and extracellular matrix. In the present study, the expression of the ALP was significantly lower in group 4 when compared to the other groups. The adaptive capability of bone tissues to the mechanical stimulations depends on the bone cells (Turner & Pavalko, 1998). Osteoblasts, the bone-forming cells are situated on the surface of bones and are triggered by mechanical stimuli *in-vitro*. Osteoblasts in cancellous bone explant models have been reported to be sensitive to mechanical stimuli from a dynamic loading and circulating perfusion bioreactor system (Hao et al., 2013). The increased ALP activity observed was congruent with the amount of new bone formation and blood capillaries and was the highest with group 3. Conversely, group 4 had the lowest percentage of new bone formation, blood capillaries as well as ALP. The highest percentage of OPN, which is a marker for the osteogenesis process, was also observed with group 3. OPN appears to be essential for the mechano-stimulation of osteogenesis in both long bones and cranial sutures. In addition, mechanical stimulus is known to induce and/or increase OPN expression in fibroblasts, pre-osteoblasts, osteoblasts, trans-chondral cells, and osteocytes (Perrien et al., 2002; Pinero et al., 1995; You et al., 2001). The results of the present study were in agreement with earlier studies that used immunostaining of ALP and OPN for the detection of new sutural bone formation. Increased ALP expression in the midpalatal suture of the mice after expansion when compared to sham groups were observed in other studies (Liu et al., 2014; Wu et al., 2015). Periosteum contains numerous cell types and precursor cells that maintain osteoblastic differentiation during bone formation and bone growth (Takushima et al., 1998). The expansive force can act on the periosteum encouraging differentiation of osteogenic progenitor cells to bone-forming osteoblasts in the midpalatal suture and expression of ALP (Hou et al., 2007). Similarly, increasing OPN expression at the margin of the midpalatal suture of rats after 100-g expansion force application (Farhadian et al., 2015).

As the current study is based on an animal model for sutural expansion, the results cannot be translated directly into clinical practice. Current miniscrew implant assisted bone-borne expanders employ an expansion regime of 0.5 mm expansion/day (group 1 protocol). Pain and discomfort associated with tooth-borne and tooth-bone-borne RME appliances have been recently compared (Feldmann & Bazargani, 2017). Both RME appliances were generally well tolerated and no significant differences in pain, discomfort, analgesic consumption and jaw function impairment were observed. While no adverse consequences were observed in any of the animals, it is uncertain if patients are able to tolerate instant expansions of up to 2.5 mm. The present study, however, yielded useful information for the refinement SARM procedures. A larger amount of initial expansion may be feasible at the time of surgery under anesthesia. This could be coupled with a shorter period of gradual expansion post-surgery reducing total treatment time.

5.3 Phase two: Piezoelectric sutural corticotomy

The use of direct forces with the maximum instant expansion to expand mature midpalatal sutures can, however, lead to high sutural stresses and variable degrees of pain sensation. To reduce these high sutural stress and pain sensation, SARME were used to facilitate transverse maxillary expansion in mature patients. The midpalatal split technique for SARME was originally described in 1938 (Brown, 1938). Converse and Horowitz subsequently proposed the use of both labial and palatal cortical osteotomies (Converse & Horowitz, 1969), while Pogrel and colleagues suggested a midpalatal cut combined with the transection of the lateral support for expansion (Pogrel et al., 1991). Instead of a single midpalatal osteotomy, other authors utilized two paramedian palatal osteotomies from the posterior nasal spine to a point just distal to the incisive canal (Bierenbroodspot et al., 2002; Koudstaal et al., 2005). Timms and Vero advocated 3 surgical stages for maxillary expansion based on the patient's age. Stage 1 (midpalatal osteotomy) is performed for patients aged 25 years or older, or younger if rapid maxillary expansion was attempted without success. Stage 2 (midpalatal and lateral osteotomies) is indicated for patients aged 30 years and older, and stage 3 (midpalatal, lateral maxillary, and anterior maxillary osteotomies) for patients aged 40 years and older (Timms & Vero, 1981). The midpalatal split or sutural corticotomy was traditionally done with burs, osteotomes or reciprocating saws between the central incisors with a soft-tissue incision (Bays & Greco, 1992).

Bone is a hard tissue and many cutting or drilling osteotomies are very rough tools. In particular, rotating devices are potentially damaging due to the production of extremely high temperatures, which can produce marginal osteonecrosis and impair bony regeneration (Kerawala et al., 1999). In recent years, the use of minimally invasive piezoelectric corticotomies was advocated to decrease the adverse side effects of conventional instrumentation (Di Alberti et al., 2010; Rana et al., 2013; Vercellotti, 2000).

The piezoelectric effect is the creation of electrical tension on some crystal and ceramic materials such as quartz to which a mechanical pressure is subsequently applied. The material in question will expand and then contract leading to an ultrasonic vibration. Also known as 'pressure electrification', it has been defined by the term 'piezo' derived from 'piezein' (Greek word) (Pavlíková et al., 2011).

The cutting of hard tissue with ultrasonic vibrations that are formed by the piezoelectric effect was first described by Catuna in 1953 and then by Volkov and Shepeleva in 1974 (Catuna, 1953; Volkov & Shepeleva, 1974). In 1981, its application was described by Aro and coworkers in orthopedic surgery (Aro et al., 1981), and Horton and colleagues in oral surgery (Horton et al., 1981). The first model of current piezoelectric devices is still being developed and heavily discussed in studies by Vercellotti and colleagues (Vercellotti, 2004; Vercellotti, 2009). Piezoelectric devices operate with the principles that are similar to the piezoelectric dental scaler devices, commonly used in the dental practice, but the ultrasonic dental scalers are not capable of cutting through hard tissues. The most innovative feature of the piezoelectric device is selective cutting. Although piezosurgery cuts mineralized tissues such as bones, it does not cut soft tissues such as vessels, nerves, and mucosa (Schaeren et al., 2008).

The phase two of the present study examined the effect of piezoelectric sutural corticotomies on accelerated bone-borne sutural expansion and compared the differences between continuous and discontinuous sutural corticotomy. The piezoelectric sutural corticotomies enhanced the outcome of accelerated bone-borne sutural expansion and significant difference in sutural separation, bone volume fraction, and new bone formation were observed between continuous and discontinuous corticotomies.

Both continuous and discontinuous corticotomies increased the potential for sutural expansion (Figure 4.10, 4.13). Accelerated sutural expansion with continuous corticotomy lead to 49.59% more sutural separation than without corticotomy. For discontinuous corticotomy, sutural separation was 34.69% above the fore mentioned treatment. Micro-CT and histologic analysis confirmed the greater extent of sutural separation in the corticotomized groups (Table 4.2). The differences in sutural separation can be attributed in part to the reduced sutural resistance in these experimental groups. Findings corroborated those of Wright on the frontonasal sutures of adult rabbits (Wright, 2015). The author concluded that mature sutures expanded with adjunctive corticotomies undergo 31% more sutural separation than sutures expanded without corticotomies. The quantum of sutural expansion was, however, much higher in our study and could be ascribed to the accelerated sutural expansion protocol comprising 2.5 mm instant (initial) Hyrax activation and effective miniscrew implant anchorage.

The higher bone volume fraction and new sutural bone formation in the corticotomized groups (i.e. groups 2 and 3) were associated with more blood capillaries and smaller amount of non-osteoid tissue (Table 4.2). Local blood supply affects the pattern of bone-forming process with bone formation occurring in regions with adequate vascularity and cartilage formation in ischemic areas (Carter et al., 1998). The development of blood vessels during the first weeks after surgery may, therefore, be the crucial factor affecting tissue regeneration. Penetrating blood vessels increase oxygen partial pressure and pH in the surrounding tissues. Low oxygen partial pressure and pH stimulate osteogenic cells to differentiate into chondroblasts leading to cartilaginous tissue formation and consequently less bone development (Brutscher et al., 1993; Kojimoto et al., 1988; Krawczyk et al., 2007). Piezoelectric osteotomy is a thermally harmless technique based on ultrasonic vibration of an osteotomic device (Vercellotti, 2003). Clinical and laboratory studies on the use of piezoelectric instruments for tooth excision and bone surgery reported ease of tooth/bone removal, higher precision, and favorable osseous response. In addition, patient discomfort appeared to be reduced, resulting in higher acceptance (Horton et al., 1981; Vercellotti et al., 2005). Furthermore, piezoelectric surgery was found to promote biological effects on odontoblast-like cells, osteoblasts, and osteogenic cells and may be potentially beneficial for osseointegration (Scheven et al., 2009). As sutural bone remodeling is enhanced with piezoelectric corticotomies, more new bone formation in the sutures is anticipated. The increased amount of new sutural bone formation observed explained the negative correlation between bone volume fraction and sutural space volume.

Corticotomies and subsequent regional acceleratory phenomenon (RAP) have also been shown to increase the cellular response at the site of injury. RAP is indicated to an increase in the rate of bone modeling and a decrease in bone density that occurs as a part of the body's natural healing process when bone is injured (Mostafa et al., 2009; Sebaoun et al., 2008). The RAP is defined as a temporary state of accelerated healing, with a regional increased in cellular activity and bone remodeling at the site of injury through the employment of osteoblasts and osteoclasts (Yaffe et al., 1994). It contains an increase in vascular perfusion, an increase in bone turnover, and a decrease in bone density, or osteopenia. The osteopenia is temporary, however, and is quickly followed by rapid osteoblastic activity and bone modeling. Due to the RAP, it has been found that the selective alveolar decortications resulted in a three-fold increase in osteoclastic activity and bone apposition at the site of injury (Sebaoun et al., 2008). Hou and colleagues demonstrated that expansion alone promoted bone resorption through increased osteoclast activity and activated proliferation of periosteal cells to form new bone and cartilage in rats (Hou et al., 2007). Since expansion alone has the capacity to induce remodeling similar to RAP, it may be plausible to increase its effect by causing surgical insult with piezoelectric corticotomies (McBride et al., 2014). Continuous corticotomy performed better than discontinuous corticotomy. The smaller bone volume fraction and new sutural bone formation with discontinuous corticotomy was associated with a lower percentage of blood vessels. Sutural areas that were not corticotomized might be subjected to large expansion forces that may rupture blood capillaries leading to less bone remodeling. In addition, RAP which normally follows corticotomy, might not occur in these regions which could decreases new bone formation.

As the piezoelectric surgery is able to enhance the biological responses of osteoblasts and osteogenic cells and could have potential benefits in osseointegration (Scheven et al., 2009). These events could explain the lower percentage of bone volume fraction and new sutural bone formation in the discontinuous piezoelectric corticotomy group (group 3).

To confirm the amount of new bone formation in the non-corticotomized and corticotomized suture we measure the intensity of ALP and OPN expression in the sutural gaps, which reflects sites of mineralized matrix deposition (Cowles et al., 1998; Perrien et al., 2002). ALP and OPN intensity were higher in the continuous corticotomy followed by discontinuous corticotomy and non-corticotomized suture indicating the advanced state of bone tissue differentiation in the corticotomy site. The increasing intensity of ALP expression in corticotomized groups designates that the using of piezoelectric instrument in sutural corticotomy leading to accelerated osteogenesis and eventually matrix deposition, which in turn increases the amount of new suture bone formation and might subsequently reduce the orthopedic relapse.

Our study had several limitations. As it was based on an animal model for sutural expansion, results cannot be directly extrapolated to clinical practice as the human palate is structurally different and larger in dimension. Accelerated bone-borne sutural expansion augmented with continuous piezoelectric corticotomy holds promise as a technique for increasing sutural expansion and reducing treatment duration in mature patients with transverse maxillary deficiency. Furthermore, an understanding of the biological processes that occur during accelerated bone-borne expansion with and without piezoelectric sutural corticotomy will provide knowledge and insight on current expansion techniques, as well as influence future treatment protocol. This will allow information and research to guide the advancement of clinical orthopedic expansion techniques, especially in those patients with severe transverse maxillary constriction.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The effects of accelerated bone-borne expansion protocols on sutural separation and sutural bone formation with/without corticotomy were investigated using micro-CT and histomorphometry/immunohistochemistry and the following conclusions were obtained:

1. The miniscrew implant assisted bone-borne modified hyrax expanders successfully expanded the midsagittal sutures for all experimental groups.
2. The amount of sutural separation was found to be directly related to the extent of instant expansion of the bone-borne RME appliance.
3. Sutural bone formation, however, correlated to the amount of instant expansion only to a critical point.
4. The expansion protocol involving 2.5 mm instant expansion followed by 0.5 mm expansion per day for 7 days resulted in the largest amount of new bone formation and was the optimal for accelerated sutural expansion.
5. When instant expansion was increased to 4 mm, sutural bone remodeling was compromised and new bone formation was decreased.
6. Piezoelectric corticotomy increases sutural separation and promotes new sutural bone formation/osteogenesis.
7. Continuous corticotomy offered better outcomes than discontinuous corticotomy.

6.2 Recommendations

While no adverse consequences were observed in any of the animals, it is uncertain if patients are able to tolerate instant expansions of up to 2.5 mm. This warrants further investigation as to the clinical outcomes and adverse effects of the proposed accelerated sutural expansion protocols. Future long-term clinical studies involving large patient samples are also needed before a conclusive protocol for accelerated maxillary expansion can be derived. By the same token, the influence of retention times and degree of relapse associated with various expansion protocols also need to be clarified.

Further studies are recommended to study the relationship between instant expansion and soft tissue reaction. Future studies are also can be done together with measurement of the amount of force applied in relation to the instant expansion.

A longer retention period is needed to confirm the favorable preliminary data for using piezoelectric sutural corticotomy. Further histological and animal studies with larger sample sizes are needed to evaluate the long-term stability of the newly formed bone.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

Published paper:

1. Akram S. Alyessary, Adrian U J. Yap, Siti A. Othman, Mohammad T. Rahman, and Zamri Radzi. **Effect of piezoelectric sutural ostecotomies on accelerated bone-borne sutural expansion.** *Journal of oral and maxillofacial surgery,* http://dx.doi.org/10.1016/j.joms.2017.08.018 (appendix G).

Accepted papers for publication:

1. Akram S. Alyessary, Adrian U J. Yap, Siti A. Othman, N Ibrahim, Mohammad T. Rahman, and Zamri Radzi. **Bone-borne accelerated sutural expansion: A micro-CT study in rabbits.** *American Journal of Orthodontics and Dentofacial Orthopedic*, (appendix H).
2. Akram S. Alyessary, Adrian U J. Yap, Siti A. Othman, Mohammad T. Rahman, Zamri Radzi, and AL-Namnam NM. **Is there an optimal initial amount of activation for midpalatal suture expansion? A histomorphometric and immunohistochemical study in a rabbit model.** *Journal of Orofacial Orthopedics,* (appendix I).
3. Akram S. Alyessary, Siti A. Othman, Adrian U J. Yap, Zamri Radzi and Mohammad T. Rahman. **Effects of non-surgical rapid maxillary expansion on nasal structures and breathing: A systematic review.** *International Orthodontics,* (appendix J).

Conference

1. Oral presenter in the 16th ANNUAL SCIENTIFIC MEETING MALSEC IADR 2017. (**Mini-implant assisted rapid sutural expansion: A micro-CT study in rabbits**).