EFFECTS OF VIBRATION FORCES ON MAXILLARY EXPANSION AND ORTHODONTIC TOOTH MOVEMENT

Mohammad Abdullah M Aldosari

Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the School of Dentistry,
Indiana University

August 2015

Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Sean Shih-Yao Liu, D.D.S., Ph.D., Chair

Jie Chen, Ph.D.

Doctoral Committee

Thomas R. Katona, Ph.D., D.M.D.

Ahmed Ghoneima, BDS, MSc, Ph.D.

December 16, 2014

Tien-Min G. Chu, D.D.S., Ph.D.

DEDICATION

To my parents, Dr. Abdullah and Dalal Shamma.

ACKNOWLEDGMENTS

I will always be grateful to Dr. Sean Liu for his continuous support, expertise and encouragement, and for being both a mentor and a role-model. I also would like to thank Dr. Jie Chen for his supervision and guidance with my projects. I would also like to extend my gratitude to Dr. Thomas Katona, Dr Ahmed Ghoneima, Dr. Tien-Min G. Chu for their support and Dr. Anderson Hara for heading my advisory committee. I am also grateful to Carol Bain for her assistance and input with all the technical aspects of my projects. Lastly I would like to thank Dr. Richard Gregory for his support throughout my PhD program.

Mohammad Abdullah M Aldosari

EFFECTS OF VIBRATION FORCES ON MAXILLARY EXPANSION AND ORTHODONTIC TOOTH MOVEMENT

Vibration forces (VF) have been shown to alter the formative and resorptive activities of bone. Studies have investigated the use of VF in applications such as the treatment of osteoporosis, bone fracture healing and implant osteointegration with favorable results. In dentistry, orthodontic tooth movement and maxillary suture expansion are common procedures typically requiring prolonged treatment durations with high relapse rates. We hypothesized that local, intermittent VF applications can enhance bone formation during rapid maxillary expansion and accelerate orthodontic tooth movement. Moreover, we also investigated expression of periostin/OSF-2, an adhesion molecule implicated in the formation of bone during maxillary suture expansion. Our results showed that intermittent VF significantly increased bone volume density of the expended palatal bone but limited the amount of palatal expansion and mineral apposition rate at the suture margins. Also, intermittent VF forces did not show statistically significant acceleration of orthodontic tooth movement but significantly enhanced bone volume density of the interradicular bone after tooth movement. Maxillary expansion was also shown to induce the expression of periostin which was proportional to the magnitude of the expansion force with increased bone mineral deposition.

Sean Shih-Yao Liu, D.D.S., Ph.D., Chair

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Dental malocclusion is a prevalent finding in the general population across all age groups (W. R. Proffit et al., 1998). In the United States, approximately 45% of children (ages 8-11), 55% of youth (ages 12-17), and 65% of adults (ages 18-50) exhibit tooth crowding or misalignment (W.R. Proffit, 2013). Furthermore, approximately 10% of this population exhibits narrow maxillae with posterior crossbites (Brunelle et al., 1996). Dental malocclusion have been associated with problems with masticatory functions (Barbosa Tde et al., 2013; Magalhaes et al., 2010), oral health (Andiappan et al., 2014; Borzabadi-Farahani et al., 2011; Masood et al., 2013), psychological and quality of life issues (Agou et al., 2008; Barbosa Tde et al., 2013; Martins-Junior et al., 2012). Depending on the severity, treatment of dental malocclusions typically requires a significant amount of time to accomplish often spanning a few years (Fisher et al., 2010; Jarvinen et al., 2004; Mavreas & Athanasiou, 2008; Parrish et al., 2011). This long treatment duration increases the risk of adverse events such as the development white spot lesions (Mizrahi, 1982; Srivastava et al., 2013), periodontal problems (McComb, 1994; Nimeri et al., 2013) and external root resorption (Rakhshan et al., 2012; Tieu et al., 2014) in addition to increased financial costs and inconvenience (Badran et al., 2014). In order to relieve moderate dental crowding and correct posterior discrepancies, rapid maxillary expansion (RME) is commonly used to widen narrow maxillae and broaden the smile arc in order to improve patients' appearances and oral functions (Habersack et al., 2007; Keim et al., 2008). However, treatment relapse often occurs because the two maxillary shelves tend to move back toward each other into the remaining unfilled suture gap after expansion. Therefore, a long period of retention for

at least 3-6 months is recommended after RME (Ekstrom et al., 1977; Hicks, 1978; Schauseil et al., 2014). A need exists for a non-invasive or a minimally invasive method to clinically increase orthodontic tooth movement (OTM) and RME efficiency to provide more predictable treatment outcomes.

In order to improve OTM or RME treatment outcomes, we need to modify the bone response to treatment. It has been well established that bone in general responds to changes in its loading environment (Turner et al., 2009). Within its physiologic limits, bone formation occurs as a response to increasing loads, but diminished loading stimuli results in lower bone formation and increased resorption (McBride & Silva, 2012; Turner et al., 1994). It is also recognized that bone responds to dynamic but not static loading, and that only a short duration of mechanical loading is sufficient to initiate a response (Turner, 2006; Turner & Robling, 2005). Also, in order to elicit an anabolic bone response, loading cycles needs to be intermittent with sufficient breaks to regain sensitivity (Robling et al., 2002b; Saxon et al., 2005; Turner, 1998). Based on these characteristics of bone tissue, previous investigations have attempted to increase bone remodeling through cyclic loading or vibrational forces (VF). It has been shown that vibrational forces (VF) effectively improve osteoporosis (Gilsanz et al., 2006; Wysocki et al., 2011), fracture healing (Kasturi & Adler, 2011) and implant osteointegration (Zhang, Torcasio, et al., 2012; Zhang, Vandamme, et al., 2012). Parameters of vibration that could be controlled includes vibration frequency, amplitude, treatment duration and application schedules, and different combinations of these parameters have been tested in a variety of uses. A few studies have attempted to investigate VF for the acceleration of tooth movement, however no studies compared the effects of different vibration frequencies or application schedules on OTM.

Also, no studies are currently available describing the effects of VF treatments applied during maxillary expansion.

A better understanding of the processes involved in bone formation and remodeling during RME will help identify possible biological targets that can be used to accelerate suture bone formation and maturation. It has been recognized that periostin/OSF-2 plays an essential role in bone remodeling during orthodontic tooth movement (Lv et al., 2014; Taddei et al., 2013; Watanabe et al., 2012). However, the role of periostin in bone formation during palatal expansion is not fully understood (Merle & Garnero, 2012) Encoded by the POSTN gene, periostin is a matricellular protein originally identified in the periosteum of bone and periodontal ligaments of mice (Horiuchi et al., 1999; Takeshita et al., 1993). It has been suggested that periostin enhances cellular adhesion and is identified as having roles in a wide range of biological processes such as tumorigenesis, arthritis, atherosclerosis, cardiac repair and embryonic development (A. Y. Liu et al., 2014). It is mainly found in collagen rich connective tissues such as the periodontal ligaments, skin cardiac valves and the periosteum (Norris et al., 2007; Romanos et al., 2014). Understanding the role of periostin in maxillary expansion might help design treatments to achieve reduced treatment times and predictable RME treatment outcomes.

The overall objective of this study is to investigate the effects of VF on maxillary expansion and orthodontic tooth movement, and to compare the effects of two different expansive forces on RME and the expression of the adhesion protein periostin/OSF-2 within the maxillary sutures. Three animal studies were designed to achieve the following specific aims:

Specific aim 1: To determine the effects of locally delivered, intermittent VF treatments on rapid maxillary expansion (RME).

Specific aim 2: To investigate and compare the effects of intermittent and locally delivered VF at two different frequencies and two different application regimens on orthodontic tooth movement (OTM).

Specific aim 3: To determine the expression of periostin/OSF-2 in the maxillary suture during expansion, and compare the effects of two levels of expansion forces on periostin expression and bone formation.

THE EFFECTS OF VIBRATIONAL FORCES SUPERIMPOSITION ON MAXILLARY EXPANSION IN RATS

Introduction

Rapid maxillary expansion (RME) is a commonly used orthodontic approach to correct transverse maxillary deficiencies. (Keim et al., 2008). The orthopedic separation of the palatal shelves induces new bone formation within the midpalatal suture resulting in a wider palatal width, which in turn alleviates issues such as dental crowding, posterior cross-bites or underdeveloped maxillae (Bishara & Staley, 1987; Schauseil et al., 2014). However, it has been demonstrated that maxillary expansion treatments undergo significant relapse, mainly due to the incomplete filling of the remaining suture gap by mature bone. Retention times of six months or more are proposed to allow sufficient bone formation and maturation within the suture to minimize RME relapse (Ekstrom et al., 1977; Hicks, 1978; Schauseil et al., 2014).

Vibrational forces (VF) has been considered as a non-pharmacological and noninvasive approach to stimulate bone formation. Positive results were reported in applications such as osteoporosis prevention and treatment (Gilsanz et al., 2006; Wysocki et al., 2011), bone fracture healing (Kasturi & Adler, 2011) and improving dental implant osteointegration (Zhang, Torcasio, et al., 2012; Zhang, Vandamme, et al., 2012). Cyclic VF loads delivered to long bone results in increased bone formation and mineral apposition rates (McBride & Silva, 2012; Turner et al., 1994). Moreover, cyclic VF has been shown to increase bone volume density of the alveolar bone (Alikhani et al., 2012). and induced

new bone growth of the craniofacial sutures (Kopher & Mao, 2003). However, no attempts have been done to study the effects of intermittent cyclic loading on bone formation during RME. The aim of this study is to investigate the effects of vibrational forces (VF) on the maxillary suture during RME in rats.

Materials and methods

Animal procedures

All animal procedures were approved by the Institutional Animal Care and Use Committee. Forty six 7-week-old male inbred rats (Fischer 344, Harlan Labs Indianapolis, IN) were used for this study. The animals were randomly assigned into four groups and received rapid maxillary expansion with vibrational force treatments (RME+VF), rapid maxillary expansion only (RME only), vibrational force treatments only (VF only), and no RME and no VF as the control, respectively.

For each animal, a mixture of xylazine (5-10 mg/kg) and ketamine (40-95 mg/kg) was injected intraperitoneally to induce anesthesia. Stainless steel coil springs were then bonded to the upper 1st and 2nd molars bilaterally using orthodontic bonding resin (3M Transbond) (Figure 1). The bonded springs were custom made from 0.014 inch stainless steel wire and calibrated to deliver 22g of expansion with 2.5mm of activation using Nano17 force gauge (ATI Industrial Automation, Apex, NC) (Figure 2). Afterwards spring placement all animals were fed with milled standard rodent diet and water ad libitum and housed under standard conditions.

Application of VF treatments

A custom made device was assembled and used for delivering VF (Figure 3). VF was applied on the hard palate three times per week for 2 weeks. Under anesthesia induced by inhalation of isoflurane, each animal was secured in the supine position with mouth opened. An actuator probe of the VF device was placed against the surface of the palate at the midpoint between the maxillary 1st molars, registering a standardized pre-load of 70 cN by the transducer. Afterwards, a VF treatment was initiated and lasted for 8 minutes with the frequency at 60 Hz and 40 μm of displacement resulting in 17.5±2.5 cN of loading applied in a sinusoidal wave pattern (Figure 4).

Calcein green (10mg/kg) and alizarin red (20mg/kg) were injected intraperitoneally as vital fluorochrome stains 11 days and 4 days prior to euthanasia. Animals were inspected and weighed three times a week. After 14 days of RME, the animals were euthanized using carbon dioxide inhalation and the maxillae were dissected, fixed and stored in a 70% ethanol solution.

Micro-CT analysis

Fixed maxillae specimens were scanned using Skyscan 1172 micro-CT system at the resolution of 8.99 μm/pixel size. The system was set at a source of 60 kV/165 μA, a rotation angle of 180° with a rotation step of 0.7°. Raw image data were then reconstructed using NRecon software (Version 1.6.9.1; SkyScan). The reconstructed images were then exported to using Ctan software (v1.13.5.1). Palatal width was measured from the bone surface adjacent the midpoint of the mesio-palatal root of the left first molar to its

contralateral counterpart on the right side. Suture width was also measured by averaging the distance between the surfaces of the sutural bones at the level of mesio-palatal root (Figure 5).

An area of interest (ROI) (1.5 mm x 0.5 mm x 90 slices) was selected from the palatal bone 200 micron lateral to the suture surface (Figure 6). Bone volume fractions, defined as bone volume / total volume (BV/TV%), and bone mineral density, indicated by mean greyscale values, were was measured and calculated using Ctan software from the ROI.

Dynamic bone histomorphometry

The dissected maxillae containing the palatal sutures were infiltrated and embedded in methyl methacrylate. Using a motorized microtome, serial sections with thickness of 4 micron were obtained in the coronal plane. Under epifluorescent microscopy, the interlabel distances between calcein and alizarin bone labels of both palatal shelves was calculated using Bioquant Osteo software (BIOQUANT Image Analysis Corporation, Nashville, TN) (Figure 7). Mineral apposition rates (MAR) were calculated by averaging inter-label distances divided by the number of days between the two vital fluorochrome label injections (um/ day).

TRAP staining for osteoclasts

Tartrate-resistant acid phosphatase (TRAP) staining was performed to visualize osteoclasts. Multinucleated TRAP-positive cells appearing in the suture and extending approximately 700 μm laterally in 2 nonconsecutive sections were measured under an inverted microscope (Leica DMI4000 B, Leica Microsystems, Bannockburn, IL) at high magnification (40×). The average ratio of the bone surface area in direct contact with osteoclasts versus the total bone surface area (osteoclast surface/bone surface; Oc.S/BS) in each ROI was calculated using the Bioquant Osteo software (Figure 8).

Statistical analysis

Two-way ANOVA with Fisher's Protected Least Significant Differences post-hoc test was used for all comparisons at the 5% level of statistical significance using Statistical Analysis System (SAS software) version 9.2 (SAS Institute, Inc., Cary, North Carolina, USA).

Results

Both RME-only and RME+VF groups had significantly higher palatal widths ($p<0.001$) (Figure 9), Oc.S/BS ratios ($p<0.001$) (Figure 10), suture widths ($p<0.001$) (Figure 11) and MAR ($p<0.001$) (Figure 12) and significantly lower BV/TV% ($p<0.001$) than VF-only and the control groups (Figure 13). The RME+VF group had significantly lower palatal width ($p<0.001$), lower MAR ($p<0.001$), and higher BV/TV ($p< 0.05$) than RME only group. When comparing VF-only and control groups, VF had no effects on

BV/TV (p=0.18), MAR (p=0.89), Oc.S/BS, or suture width (Figures 10, 6). Neither RME (p=0.57) nor vibration (p=0.23) had a significant effect on mean greyscale values (Figure 14).

Discussion

The present study aimed to investigate the effects of superimposed mechanical vibrational forces during maxillary expansion in rats. In this study, the application of intermittent VF during maxillary expansion reduced RME resulting in narrower palatal widths after 2 weeks when compared to maxillary expansion without VF. However, applying VF resulted in significantly higher bone volume of the expanded palatal bones, which is consistent with studies observing higher bone volume fractions under cyclic load stimulation (Alikhani et al., 2012; Kasturi & Adler, 2011; Omar et al., 2008). Newer, less mineral-dense bone would produce lower mean grayscale values due to lower x-ray attenuation (Bouxsein et al., 2010; Ritman, 2011; Sheets et al., 2013). In this study, no changes in mean grayscale values were observed. Thus, the higher bone volume fraction BV/TV of the expanded sutural bones under VF may not be due to increased bone formation but as a result of reduced osteoclast numbers or activity. Although not statistically significant, the osteoclast surface to bone surface ratio in the RME+VF group was lower, which could be a plausible explanation for the higher bone volume fractions of the RME+VF compared to RME-only. This deduction is supported with studies that hypothesized that VF would has an inhibitory effect on osteoclasts (E. Lau et al., 2010; Ozcivici et al., 2010; Zhou et al., 2014).

The suture widths were similar in both expansion groups. Thus, the difference in palatal width can be attributed the difference in the of new mineral apposition rate at suture edges. The mineral apposition rate at the suture margins of the RME+VF group was lower when compared the RME-only group. It has been demonstrated that the amount of new bone formation during suture expansion at the suture margins is controlled the by the amount of suture separation (S. S. Liu et al., 2010; S. S. Liu et al., 2011). Given the similarity of the amount of suture separation between the expansion groups, the reduction of the MAR in the RME+VF group can be attributed to the effect of VF treatments. This finding is contrary to studies that showed that vibrational loading increased bone formation in long bones, primarily due to different mechanisms of stimulating bone formation (Sun et al., 2014; Wang et al., 2014) Also, these studies observed bone formation under isolated VF loading, which did not include the constant, bone formative, tensile force delivered through suture expansion. It has been established that maxillary expansion causes prolonged stretching of the connective tissue fibers resulting in bone formation at the opposing surfaces of the suture (Katebi et al., 2012; Schauseil et al., 2014). In contrast, long bone cyclic loading creates transient areas of compression and tension that results in sporadic burst of adaptive remodeling (McBride & Silva, 2012; Pearson & Lieberman, 2004). In this study, bone formation of the RME+VF group was influenced by a combination of stretched connective tissue fibers and VF stimulation.

Many studies have investigated whether low-magnitude VF with high frequency can affect bone remodeling or bone cell activities. The VF frequency ranged from 1Hz to 200Hz with a wide range of load magnitude and loading regimens. No current literature is available directly comparing with the present study due to the variety of VF parameters

and loading regimens. In addition, no attempts have been made in the past to study the effects of VF during suture expansion. The VF frequency of 60 Hz delivered three times a week was chosen for this experiment which is a frequency commonly used to provoke anabolic responses in bone when subjected to loads (Alikhani et al., 2012; de Oliveira et al., 2010; Reyes et al., 2011). The higher frequency allows for a high number of load cycles/ day delivered per animal within a practical timeframe. Loading regimens in studies varied form daily treatments to one treatment per week. Rubin et al. and others have found that the bone response to loading can be increased progressively given that enough "breaks" were inserted between loadings to regain sensitivity (Robling et al., 2001; Robling et al., 2002a, 2002b). In this study three VF treatments per week were delivered thus enabling sufficient number of treatments while allowing rest periods for bone to regain sensitivity.

Many animal studies have attempted to increase bone formation during maxillary expansion. Local administration of medications (Uysal, Amasyali, et al., 2009; Uysal et al., 2011), stem cell injections (Ekizer et al., 2014), low level laser therapy (Cepera et al., 2012; da Silva et al., 2012), and systemic medications (Uysal, Ustdal, et al., 2009) have been attempted with varying levels of success. It is advantageous to develop a non-invasive non pharmacologic approach to improve RME treatment outcomes. In this study we were able to demonstrate that applying local stimulation using vibrational forces can cause significant changes on the maxillary expansion process. However, at our tested conditions it resulted in a decrease of palatal expansion, although an enhancement of the bone volumes of the palatal bones was observed. Further investigations exploring different frequencies, loads and routines of the VF is needed. A lower VF frequency such as 30 Hz, fewer applications per week or lower loads of the VF may provide an improved result by maintaining bone

volume in the palatal bones while allowing more bone mineral apposition within the sutures.

EFFECTS OF INTERMITTENT VIBRATIONAL FORCES ON ORTHODONTIC TOOTH MOVEMENT

Introduction

Orthodontic tooth movement (OTM) is achieved by alveolar bone resorption occurring in the compression side and bone formation in the tension side of effected teeth. This dynamic nature of bone allows for clinical orthodontic treatments to occur, although it is a process which normally takes years to achieve (Fisher et al., 2010; Jarvinen et al., 2004; Mavreas & Athanasiou, 2008; Parrish et al., 2011). Increasing the rate of bone turnover may result in shorter treatment times, thus potentially minimizing the occurrence of adverse effects from orthodontic treatment. Previous studies have attempted to accelerate OTM through the application of vibrational forces (VF). Nishimura et al (Nishimura et al., 2008) demonstrated that the application of 60 Hz VF to maxillary molars in a rat model significantly increased the rate of OTM. They used expansion springs to achieve tooth movement which could have exaggerated the result through skeletal expansion rather than tooth movement. Kalajzic et al. (Kalajzic et al., 2014) found that VF of 30 Hz significantly reduced the rate of OTM, but no attempt was done to confirm constant cyclic load was being delivered to the target tooth. The aim of this present study is to investigate the effects of intermittent VF on OTM in addition to comparing two different vibrational frequencies (30Hz and 60Hz) and two different weekly application regimens on OTM while attempting to overcome the limitations of previous experiments.

Materials and methods

Animal procedures

All animal procedures were approved by the Institutional Animal Care and Use Committee. Thirty two male Fischer 344 inbred rats at 16 weeks old were purchased from Harlan Labs (Indianapolis, IN - USA) and housed under standardized conditions. After acclimation, animals were anesthetized using a mixture of xylazine (5-10 mg/kg) and ketamine (40-95 mg/kg) injected intraperitoneally. Orthodontic tooth movement (OTM) was achieved by using nickel titanium closed springs (G&H) 8 mm in length, 0.019 in lumen and 0.003 in diameter measured and calibrated to deliver 6 cN with 4 mm activation. The springs were attached to a 0.008 inch stainless steel ligature wire that was tied around the left maxillary fist molars and secured with orthodontic bonding resin (3M Transbond). The other end of the spring was also tied with ligature wire and bonded to the maxillary incisors. Right side maxillary 1st molars served as the controls (non-OTM side) (Figure 15). Afterwards the animals were randomly divided into four groups (n = 8). The first group underwent only orthodontic tooth movement (OTM-only), the second group underwent orthodontic tooth movement and also received vibration force (VF) treatment at 30 Hz twice a week, the third group had orthodontic tooth movement and also received VF treatment at 60 Hz twice a week, the fourth group underwent orthodontic tooth movement and had VF treatments at 60 Hz once a week.

Application of VF treatments

Under isoflurane anesthesia, each animal was placed in the supine position on a test bed with a mouth prop to keep the mouth open (Figure 16). An actuator probe was then be lowered into the oral cavity until the tip of the probe engages the occlusal fossa of the maxillary 1st molar, registering a pre-load of 1 N by the test bed transducer. The transducer reading is then zeroed and VF treatment would commence for 8 minutes, delivering either 30 Hz or 60 Hz VF frequency at 20 μm of displacement providing 17.5±2.5 cN of load in a sine wave form (Figure 17). These treatments were performed either once a week or twice a week according to group designation for a total of 4 weeks, with the exception of the OTM-only group which received no VF treatments.

The animals were fed ground standard diet and water ad libitum and were inspected and weighed three times a week. As the incisors erupted, springs were repositioned to ensure constant forces and were adjusted accordingly. After 4 weeks of OTM, the animals were euthanized using CO_2 inhalation and the maxillae were dissected, fixed and stored in a 70% ethanol solution.

Micro-CT analysis

Fixed maxillae were scanned using Skyscan 1172 micro-CT system at the resolution of 6.57 μm/pixel size. The system was set at a source of 60 kV/165 μA, and a rotation angle of 180° with a rotation step of 0.5°. Raw image data were then reconstructed using NRecon software (Version 1.6.9.1; SkyScan). The reconstructed images were then exported to using Ctan software (v1.13.5.1). Tooth movement due to OTM was defined as the interproximal distance between the 1st and 2nd molars, from the most mesial point of

the 2nd molar to the most distal point of the 1st molar on the same side along the line of tooth movement in the sagittal plane (Kalajzic et al., 2014) (Figure 18). Bone volume fraction is defined as bone volume / total volume (BV/TV %) and was measured using Ctan software. For measuring interradicular BV/TV, a region of interest (ROI) was defined as a cylindrical ROI 550 μm in diameter and 591 μm (90 slices) in height situated within the intra-radicular bone between the roots of the maxillary 1st molar (Figure 19). The interradicular bone volume fraction was then calculated from the ROI along with the mean greyscale values for the interradicular bone areas for both right and left maxillary 1st molars (Figure 20).

TRAP staining for osteoclasts

Tartrate-resistant acid phosphatase staining was performed to visualize osteoclasts. Multinucleated TRAP-positive cells appearing against alveolar bone were quantified under an inverted microscope (Leica DMI4000 B) at high magnification (40×). The ratio of positively stained osteoclast in direct contact with bone surface area adjacent to the mesial root versus the total bone surface area (osteoclast surface/bone surface; Oc.S/BS%) in each ROI was calculated using the Bioquant Osteo software (BIOQUANT Image Analysis Corporation, Nashville, TN).

Statistical analysis

The effect of vibration on displacement was analyzed using one-way ANOVA. Statistical analysis was performed using a repeated measures ANOVA, with side as the repeated factor with an unstructured variance/covariance matrix, and fixed effects for side, group, and the side-by-group interaction. A 5% level of statistical significance was applied. All analysis were performed using Statistical Analysis System (SAS software) v.9.2.

Results

A total of five animals which had springs fail over the pe2riod of the experiment were excluded from the study, resulting in a total of at least 6 animals per group. Similar amounts of tooth movement were achieved in all groups with no group significantly different at the 5% level (Figure 21). An increased tendency in tooth movement rate was observed in the group receiving VF at 60 Hz once a week, demonstrating a higher distance of molar separation although not statistically significant ($p = 0.054$). The molars on the contralateral non-OTM side showed no separation and remained in contact for all groups at the conclusion of the study.

On the left maxillary 1st molars (OTM side), bone volume fractions (BV/TV %) were significantly higher in the groups receiving 30 Hz VF twice/ week or 60Hz VF once/week in the interradicular area of left 1st molar, compared to OTM-only group ($p<0.01$) (Figure 22). There were no differences in the interradicular bone volume fractions on the non-OTM side between treatment groups. In the OTM-only group, bone volume fraction was significantly lower in the interradicular area of the 1st molar on the OTM side compared to its counterpart 1st molar without OTM ($p<0.05$). For the groups receiving VF,

there were no significant differences in bone volume fractions in the interradicular bone area of the 1st molars when comparing OTM and non-OTM sides.

The mean greyscale values of the interradicular bone of the 1st molars were significantly lower for the OTM sides than the non-OTM sides in the groups receiving 30 Hz twice/ week, 60 Hz twice/ week and 60 Hz once/week ($p<0.01$) but not for the OTM-only group ($p=0.3716$). There were no differences in interradicular mean greyscale values among groups for the 1st molars with OTM ($p=0.3998$) or the contralateral controls without OTM ($p=0.6700$) (Figure 23).

In the direction of tooth movement, osteoclast surface to bone surface ratios (OcS/BS) values were 9.5±6%, 10.2±5.3%, 8.7±4.2% and 8.9±6.8% for the control, 30Hz twice/ week, 60 Hz twice/week and 60 Hz once/ week groups respectively. Differences were not statistically significant between groups ($p>0.05$) (Figure 24).

Discussion

Bone resorbs adjacent to the periodontal ligament subjected to compression and new bone deposits adjacent to the periodontal ligament subjected to tension (Henneman et al., 2008; Zainal Ariffin et al., 2011). Increasing the bone remodeling process might conceivably accelerate OTM, leading to reduced treatment durations (Nimeri et al., 2013). The aim of this study was to acertain and compare the effects of two different frequencies and application regimens of locally delivered VF on OTM. The frequencies of 30 Hz and 60 Hz were chosen for this study as they are most commonly used in dental loading studies, in addition to 30 Hz also being the frequency used in a

FDA approved device for OTM (OrthoAccel Technologies, Inc, Bellaire, TX) (Alikhani et al., 2012; Kalajzic et al., 2014; Nishimura et al., 2008). The number of occurences of cyclic loading applications per week varied among studies, ranging from once a week to daily applications (Kono et al., 2012; Raab-Cullen et al., 1994; Sun et al., 2014; Turner et al., 1994). In this study we compared once weekly and twice weekly VF treatments. According to Robling et. al. (Robling et al., 2002b), Saxon et.al. (Saxon et al., 2005) and others, bone will respond to loading stimulations given that a sufficient "break" period is provided for bone to recover its "sensistivity" to respond to further stimulations. Therefore, providing VF treatments once or twice weekly is within this principle.

No significant differences in the amount of orthodontic tooth movement between study groups were observed, although animals which underwent VF treatments given at 60 Hz once/ week tended to have an increased orthodontic tooth movement after 4 weeks, although not statistically significant ($p=0.054$). High variability was observed in the tooth movement measurements of all groups. Similar variability has been previously reported (Kalajzic et al., 2014; Madan et al., 2007). However, this variability could be due to the lighter forces delivered though the more delicate sized nickel titanium coiled springs used in this study (6 grams used vs. 25g or more in other studies). Although lighter forces are generally considered favorable for tooth movement, the lighter springs would have been more sensitive to local anatomical variation between animals such as the variation in the shape and size of the maxillary incisors. Another source of variation could be the different rates of incisor eruption which can affect the amount of activation of the attached coiled springs, in addition to the changes in forces attained with spring adjustments. Also, the relatively longer duration of OTM in this study at 4 weeks may have magnified the chance

of variability in responses between animals. A study of shorter duration with heavier, more rigid springs could overcome this problem.

Kalajzic et. al. (Kalajzic et al., 2014) found that applying VF at 30 Hz twice weekly in rats inhibited OTM compared to the OTM only group. This is in contrast to our findings, as no inhibition was observed as a result to VF treatments at either 30 Hz or 60 Hz. This disparity could be due to the lower coil spring forces used to orthodontically move the teeth and the lighter vertical VF forces used in the current study compared to the study by Kalajzic (Gonzales et al., 2008; Kalajzic et al., 2014). Yee et al. (Yee et al., 2009), Weltman et al. (Weltman et al., 2010) and others have described that using lighter forces when moving teeth is advantageous with more predictable results (Barbagallo et al., 2008; Karadeniz et al., 2011; Kohno et al., 2002). Also, it has been shown that the amount of force delivered during cyclic loading is an important factor in determining the effects of VF on bone remodelling (Cullen et al., 2001; Duncan & Turner, 1995). The combination of the lighter coil spring forces along with the lighter vibrational loading may have resulted in the different outcome observed in our current study compared to the study by Kalajzic and coworkers.

The osteoclast counts on the surface of the bone directly in the path of movement was similar in all groups, which correlates with the simlar amounts of tooth movement. It was noteworthy that although amounts of OTM were similar in all goups, VF treatments at either 30 Hz twice a week or 60 Hz once a week preserved the bone volume within the intraradicular bone of the molars being moved to levels similar to their non-OTM sides. Alikhai et al(Alikhani et al., 2012) demonstrated that vibrational loads at 30 Hz and 60 Hz applied daily to rat molars increased bone volume fractions in the supporting bone. In this

study the effect was also observed during orthodontic tooth translation, and this effect was achieved while delivering only one or two VF treatments per week. In the OTM-only group, the amount of bone volume in the radicular area of the first molar on the tooth movement side was signifiacntly lower when compared to the non-OTM side after 4 weeks. Also, mean grayscale values were used to indirectly compare mineral density of the bones through x-ray attenuation (Bouxsein et al., 2010). Despite having similar amounts of bone volumes, the intra-radiclar bone in the OTM-sides had lower mean greyscale values compared to the non-OTM sides in the groups which received VF treatments. This indicates that the intra-radicular bone in the vibration groups was newer, less mineral dense bone which was formed as a consequence of VF treatments.

A disadvantage of this study is the reduced sample size due to spring failures, which affected the statistical power. Nonetheless, further studies are needed to explore other frequencies and loading schedules in order to ascertain the best parameters for a clinically effective, non-invasive method for orthodontic tooth movement.

PERIOSTIN/OSF-2 EXPRESSION DURING MAXILLARY SUTURE EXPANSION IN MICE

Introduction

Transverse deficiency of the maxilla is a frequent skeletal problem typically corrected clinically using rapid maxillary expansion (RME) (Keim et al., 2008). The orthopedic separation of the palatal shelves induces new bone formation within the midpalatal suture resulting in a wider palatal width, which in turn alleviates issues such as dental crowding, posterior cross-bites or underdeveloped maxillae (Bishara & Staley, 1987; Schauseil et al., 2014). However, it has been demonstrated that maxillary expansion treatments undergo significant relapse, mainly due to the incomplete filling of the remaining suture gap by mature bone. Retention times of six months or more are proposed to allow sufficient bone formation and maturation within the suture to minimize RME relapse (Ekstrom et al., 1977; Hicks, 1978; Schauseil et al., 2014).

To reduce RME relapse, it would be beneficial to identify possible biological targets that can be used to accelerate suture bone formation and maturation. Periostin/OSF-2 is a matricellular protein necessary for normal skeletal bone formation and maturation (Merle & Garnero, 2012; Rios et al., 2005). It is present in abundance in human and murine periosteum and periodontal ligaments, in addition to tissues subjected to mechanical stresses such as tendons (Merle & Garnero, 2012; Romanos et al., 2014). Periostin have been found to have role in osteoblast differentiation and proliferation and a biomarker for new bone formation (Merle et al., 2014). Several studies have investigated the potential for

growth factors such as BMP-2 (S. S. Liu et al., 2013) and TGF-β (Sawada & Shimizu, 1996) on improving bone formation during suture expansion, but the limited successes or the occurrence of complications drives the search for alternative biological targets (Cowan et al., 2005; Walker & Wright, 2002). Although it has been demonstrated that periostin/OSF-2 plays an important role in bone remodeling during orthodontic tooth movement (Lv et al., 2014; Taddei et al., 2013; Watanabe et al., 2012), the role of periostin in bone formation during palatal expansion is not fully understood (Merle & Garnero, 2012). The aim of this study is to investigate the expression of periostin in the maxillary suture of mice during expansion, and compare the effects of two levels of expansion forces on periostin expression and bone formation.

Materials and methods

Animal procedures

All animal procedures were approved by the Institutional Animal Care and Use Committee. Sixty inbred 6-week-old CB57/6J male mice were randomly assigned into one of three groups. Two groups underwent maxillary expansion using custom made appliances delivering either 10g or 20g of force and the third group served as a control group with no expansion.

Maxillary expansion was achieved by using custom-made expanders made using 0.010” and 0.012” nickel titanium (NiTi) wires (G&H, Franklin, IN) designed to deliver 10g, and 20 grams respectively with 2 mm of activation. A mixture of xylazine (5-10 mg/kg) and ketamine (40-95 mg/kg) was injected intraperitoneally to induce anesthesia.

Afterwards, the springs were bonded to the upper 1st and 2nd molars bilaterally using orthodontic bonding resin (3M Transbond) (Figure 25). Animals were fed milled standard rodent diet and water ad libitum and were checked and weighed daily. The animals were given Calcein (30 mg/kg) on day 1 and Alizarin complexone (50 mg/kg) intraperitoneally on day 11 post placement for fluorescent bone labeling. Animals were euthanized 14 days after placement of springs through carbon dioxide inhalation. The maxillae were then dissected and fixed with 10% formalin.

Micro-CT analysis

Fixed maxillary bones were scanned using Skyscan 1172 micro-CT system at the resolution of 13.8 μm/pixel size. The system was set at a source of 60 kV/165 μA, and a rotation angle of 180° with a rotation step of 0.7°. Raw image data were then reconstructed using NRecon software (Version 1.6.9.1; SkyScan). The reconstructed images were then exported to using Ctan software (v1.13.5.1). Suture width was measured by measuring the average distance between the surfaces of the two opposing suture margins of the palatal bones of ten image slices taken at the level of disto-palatal roots of the 1st molars. Bone volume fractions of the palatal bone (BV/TV%) were quantified by averaging the bone volume fraction values of ten image slices covering the palatal bone from the surface of the disto-palatal root of the 1st molar to the sutural margin.

Immunohistochemistry

For immunohistochemistry, dissected maxillae were embedded in paraffin and sections of 5 μm thickness in the coronal plane were obtained using a microtome. Peroxidase in the sections was inactivated and afterwards blocked with 1.5% normal rabbit serum blocking solution (Vector Laboratories, Burlingame, CA) for 30 minutes and then incubated overnight at 4^{o} C with goat anti-mouse periostin/OSF-2 polyclonal IgG antibody (Cat#AF2955, R&D Systems, Minneapolis, MN) at 1:500 dilution. Periostin-OSF-2 antibody stain was then visualized using an avidin-biotin complex immunoperoxidase kit (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA) and a 3,3′-Diaminobenzidine (DAB) substrate kit (Sigma, St. Louis, MO, USA). Mouse long bone longitudinal section with periosteal and endosteal surfaces was used as positive control tissue, and negative control sections were processed by omitting the primary antibody. Sections were then counter-stained with 0.2% Methyl Green and microphotographs were taken under standardized settings using an inverted microscope (Eclipse TS1000, Nikon, Tokyo, Japan). Semi-quantification of periostin expression was performed by evaluating positive DAB staining in connective tissues within the boundaries of the maxillary suture and the signal intensity was stratified as strong (+++), moderate (++), weak (+) or no staining (-).

Dynamic bone histomorphometry

The dissected maxillae were infiltrated and embedded in methyl methacrylate. Using a motorized microtome, serial sections with thickness of 5 microns were obtained in the coronal plane. Under epifluorescent microscopy, the inter-label distances between

calcein and alizarin bone labels of both palatal shelves were measured using Bioquant Osteo software (BIOQUANT Image Analysis Corporation, Nashville, TN). Mineral apposition rate (MAR) was calculated by averaging inter-label distances divided by the number of days between the two vital fluorochrome label injections (um/ day).

Statistical analysis

Mantel-Haenszel Chi-square test and One-way ANOVA followed by Bonferroni's post-hoc tests were performed at $p<0.05$ significance for all statistical tests. All statistical analyses were performed using the Statistic Package for Social Study (SPSS) software package version 19 (SPSS Inc., Chicago, IL, USA).

Results

Suture width was verified using micro-CT images, indicating the distance of the suture gap between the two suture margins (Table 1). The suture width after two weeks of expansion was significantly wider (413±98μm) in the 20g group than the 10g group (247±116 μm), and both the 20g and 10g groups were significantly wider than the control group (92±8μm) ($p<0.05$). Bone volume fraction of the palatal bone in the 20g group (77±8.5%) was significantly lower than the 10g group (85.7±5.5%) and the controls (89.4±2.6%) ($p<0.05$), but the 10g group was not significantly different from the control group.

Periostin expression intensity was higher in the 20g group than the 10g group followed by the control group, with all groups significantly different from each other ($p<0.05$) (Table 1). The signal was observed on the surfaces of the palatal bone suture edges and along the bundles of connective fibers within the suture area of expanded sutures (Figure 27). In the controls, the signal was sparse within the suture connective tissue with most of the signal confined to the periosteum of the palatal shelves at oral and nasal borders of the sutures, and little to no periostin expression was found directly against the suture bone surfaces in controls.

The mineral opposition rate (MAR) was significantly higher (4.5 ± 2.2 μm/day) in the 20g group than the 10g group (2.7 ± 1.2 μm/ day) which was significantly higher than the control group (1±0.77 μm/ day) ($p<0.05$)(Figure 31).

Discussion

Treatment relapse after maxillary expansion remains a significant clinical occurrence (Romanyk et al., 2010; Vargo et al., 2007). This is mainly due to incomplete filling of the suture gap with new bone after expansion. It has been suggested that periostin has the ability to recruit and attach osteoblasts to the bone surface (Bonnet et al., 2009; Kashima et al., 2009; Merle & Garnero, 2012). In this study, we observed that periostin/OSF-2 is highly expressed in the palatal suture during maxillary expansion. Periostin expression was found predominantly within the intercellular matrix along the fibrous connective tissues bridging both ends of the suture, and also found directly against the surfaces of the opposing suture shelves (Figure 3). This study also demonstrated that

the intensity of the periostin expression was associated with the amount of expansion force, which positively correlates with the bone mineral opposition on the surfaces on the sutures. In non-expanded controls, immnunohistochemical staining revealed the presence of periostin only along the periosteum and the nasal and oral boundaries of the suture, with sparse expression within the suture connective tissues.

Liu et al. (S. S. Liu et al., 2010; S. S. Liu et al., 2011) found that the amount of bone formation at the mid-sagittal suture of rabbits was proportional to the amount of suture separation and the force used. In the present study, the higher force used to expand the maxillae resulted in significantly wider sutures than those achieved with lower forces, with more bone mineral deposition at the suture margins. However, the higher expansion force resulted in a significant decrease of the bone volume fraction of the palatal bones by 12.1% when compared to controls.

Periostin is an extracellular matrix protein expressed in connective tissues subjected to stress like the periodontal ligaments, heart valves and tendons (Conway et al., 2011; Kruzynska-Frejtag et al., 2001; Rios et al., 2005; Yoshiba et al., 2007). It was also observed to have a role in the normal development of the embryonic skeleton and dental growth, with periostin deficient mice displaying defective skeletal development (Rios et al., 2005). It has been suggested that periostin promotes osteoblast functions through multiple interactions. Through the αvβ3 integrin expressed by osteoblasts, periostin activates the FAK and Akt/PKB pathways leading to cell migration and increased osteoblast survival (Butcher et al., 2007; S. S. Liu et al., 2011; Ouyang et al., 2009). Also it functions by binding to non-integrin receptors such as the Notch receptors on the surface of osteoblasts up-regulating the expression of Notch and Bcl-xl which also prolongs cell survival,

especially under mechanical stresses (Merle & Garnero, 2012; Tanabe et al., 2010). Additionally, it also hypothesized that under mechanical stress periostin is up-regulated through the Wnt/ β-catenin, stimulating bone formation (K. H. Lau et al., 2006; Robinson et al., 2006; Romanos et al., 2014).

The findings of the current investigation suggests that periostin may enhance bone formation at the suture margins during expansion. This creates the possibility of improving clinical outcomes of RME by manipulating periostin levels at the suture, thus accelerating bone formation and minimalizing treatment relapse. This result encourages further exploration of the possible roles of periostin in the RME process.

CONCLUSIONS

1. Intermittent VF under the tested experimental conditions significantly reduces maxillary expansion with less bone mineral deposition at the suture margins. Intermittent VF enhances palatal trabecular bone resulting in higher bone volume fractions during expansion, but it does not affect mineral bone density with or without RME.

2. Vibrational forces delivered at 30Hz twice weekly or 60Hz once a week during OTM maintains bone volume in the intra-radicular bone of the orthodontically moved teeth through increased bone formation compared to OTM only. However, a larger sample size is needed to ascertain the effects of VF on the acceleration of OTM.

3. Periostin/OSF-2 is highly expressed in the maxillary suture during expansion. The intensity of periostin expression is proportional to the force of suture separation with increased bone mineral deposition within the suture.

CLINICAL EXTRAPOLATION

Both orthodontic tooth movement and rapid maxillary expansion rely on the physiological responses of surrounding bone structures to occur. In this study, intermittent vibrational forces have demonstrated the ability to modify the bone response to maxillary expansion. Under the tested VF parameters, it reduced the amount of palatal width and new bone deposition, but maintained palatal bone volume. These results revealed the possibility of an ideal VF treatment to accelerate bone formation during clinical RME and demonstrated that VF has the ability to maintain the bone volumes surrounding the teeth during OTM, without affecting the amount of tooth displacement. This effect of VF treatment may potentially reduce clinical relapse following orthodontic treatment due to increased bone remodeling, or maintain bone for those at risk of further bone loss during OTM. The increased expression of periostin within the expanded palatal sutures correlates with increased expansion forces and bone formation. Clinically modifying the local amounts of periostin expression may lead to improved bone formation at the suture and thus better clinical outcomes. Our results establish a foundation for further examination of the possible roles of periostin in RME treatments.

TABLES AND FIGURES

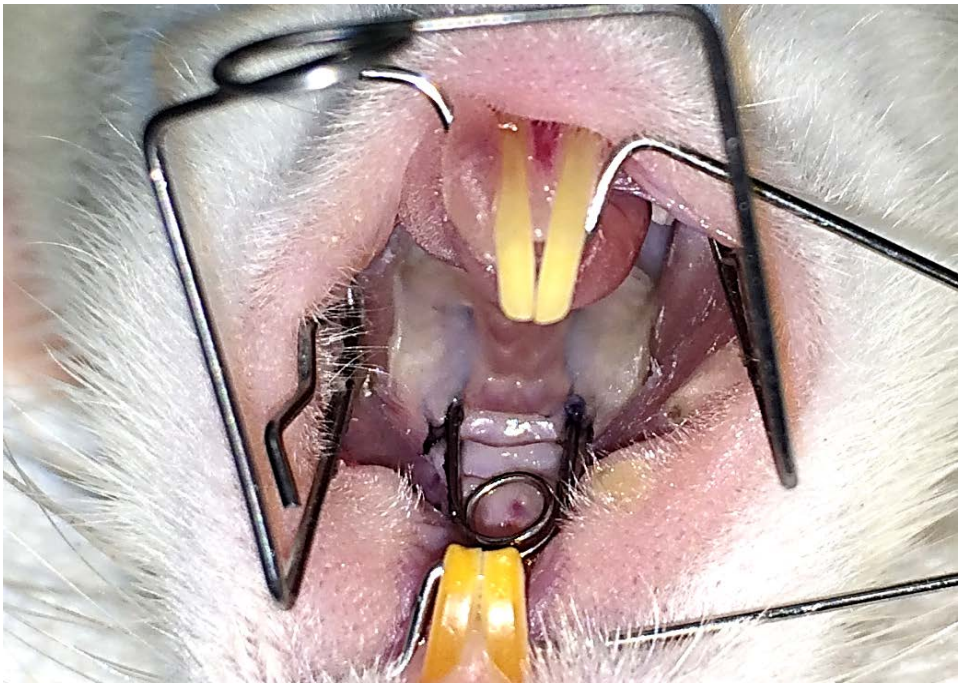


Figure 1. Custom made RME device bonded in position

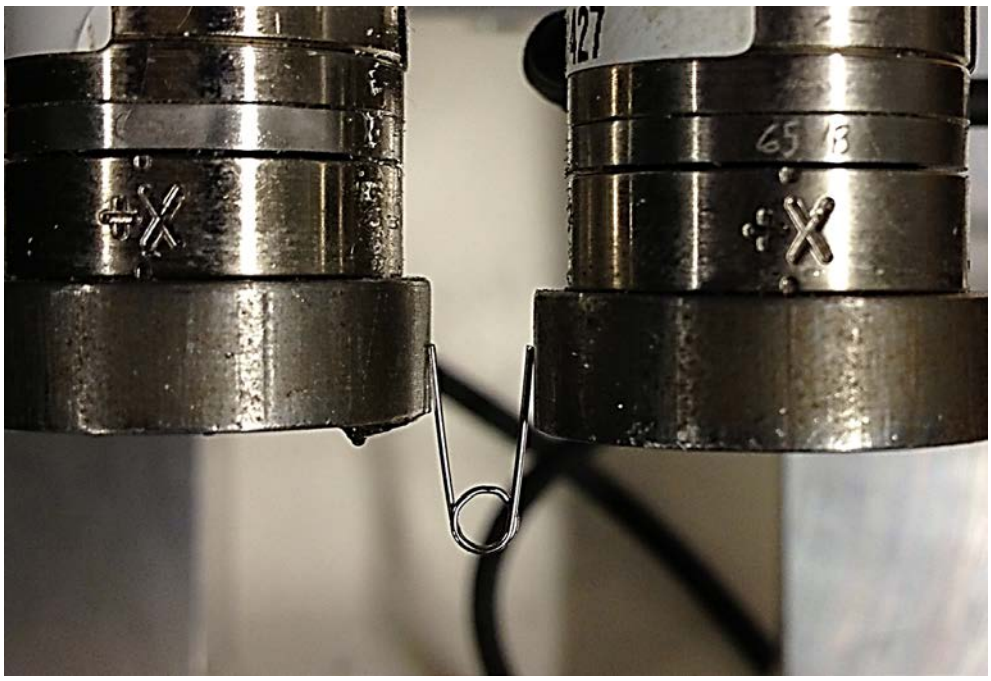


Figure 2. Spring calibration using Nano17 force gauge (ATI Industrial Automation, Apex, NC)

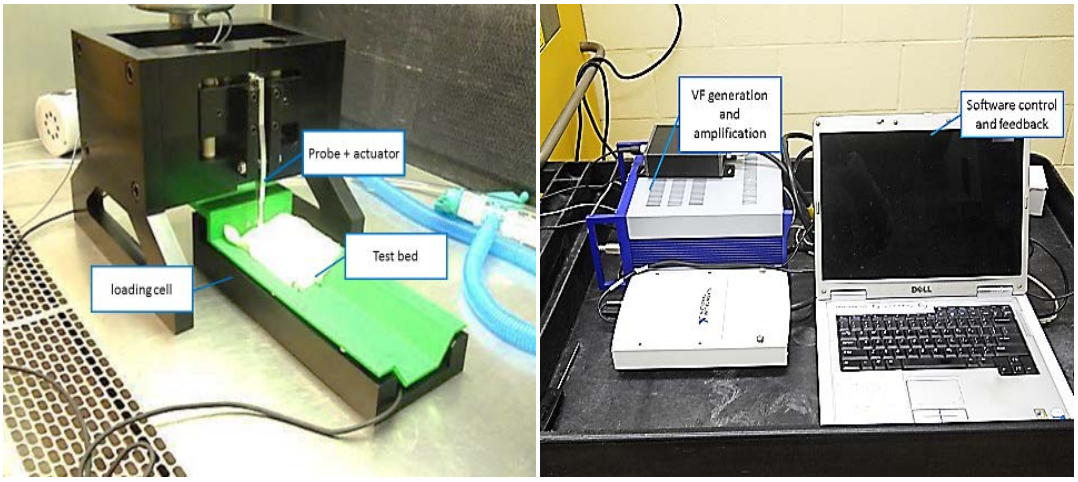


Figure 3. Components and set-up of the VF delivery apparatus.

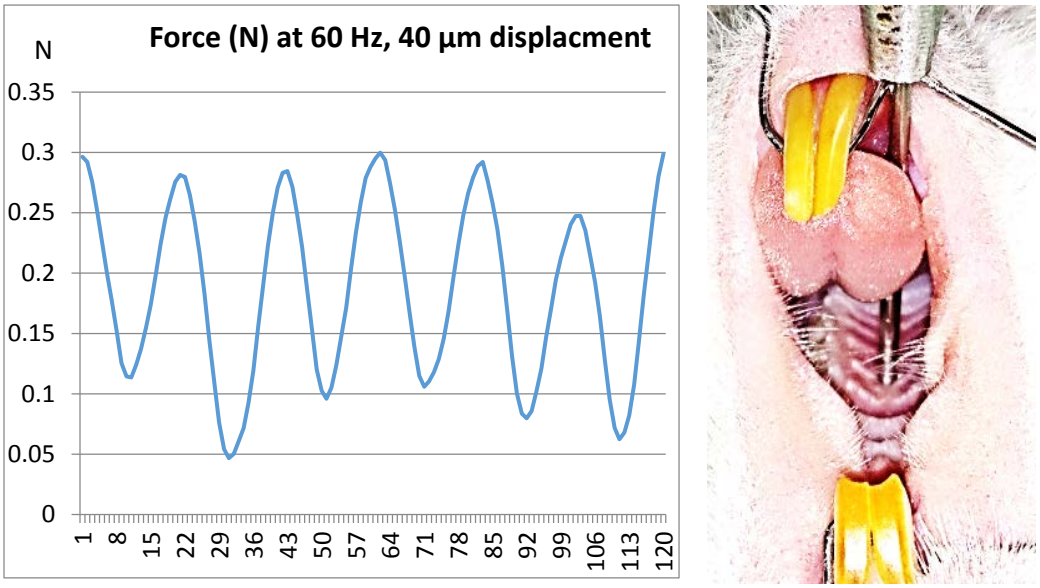


Figure 4. Typical transducer readings during VF treatments. Graph represents a 100 millisecond window on the y-axis, showing six peaks of a 60 Hz waveform (left). Intra-oral photograph showing the actuator tip delivering VF over the palatal suture (right).

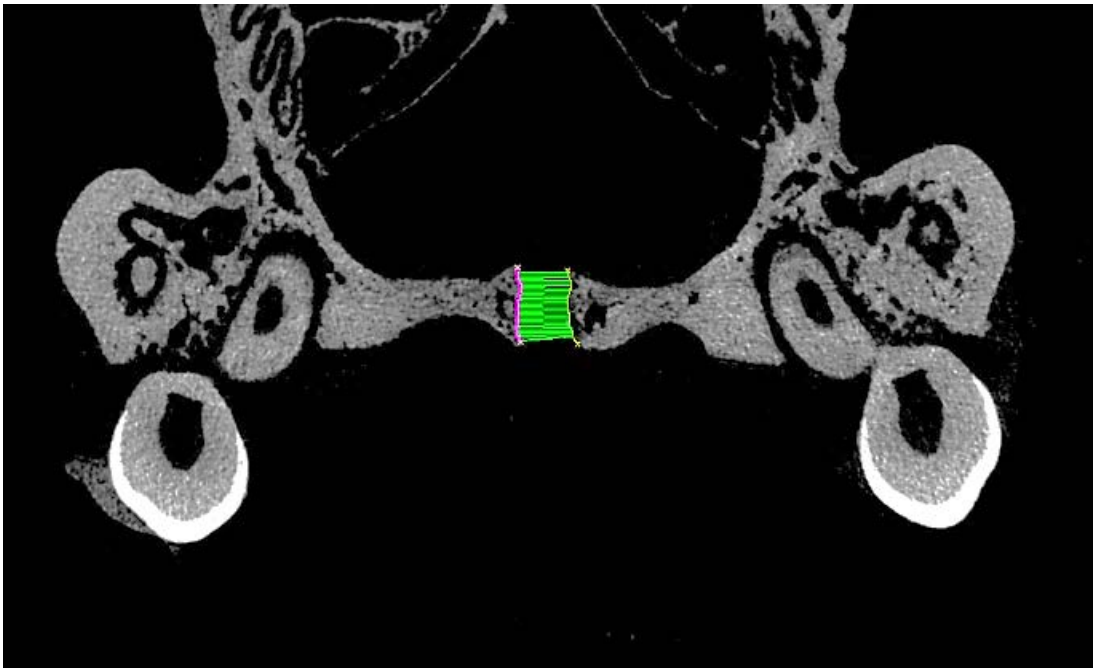


Figure 5. A micro-CT image showing expanded palatal bones with the mid-palatal suture highlighted in green.

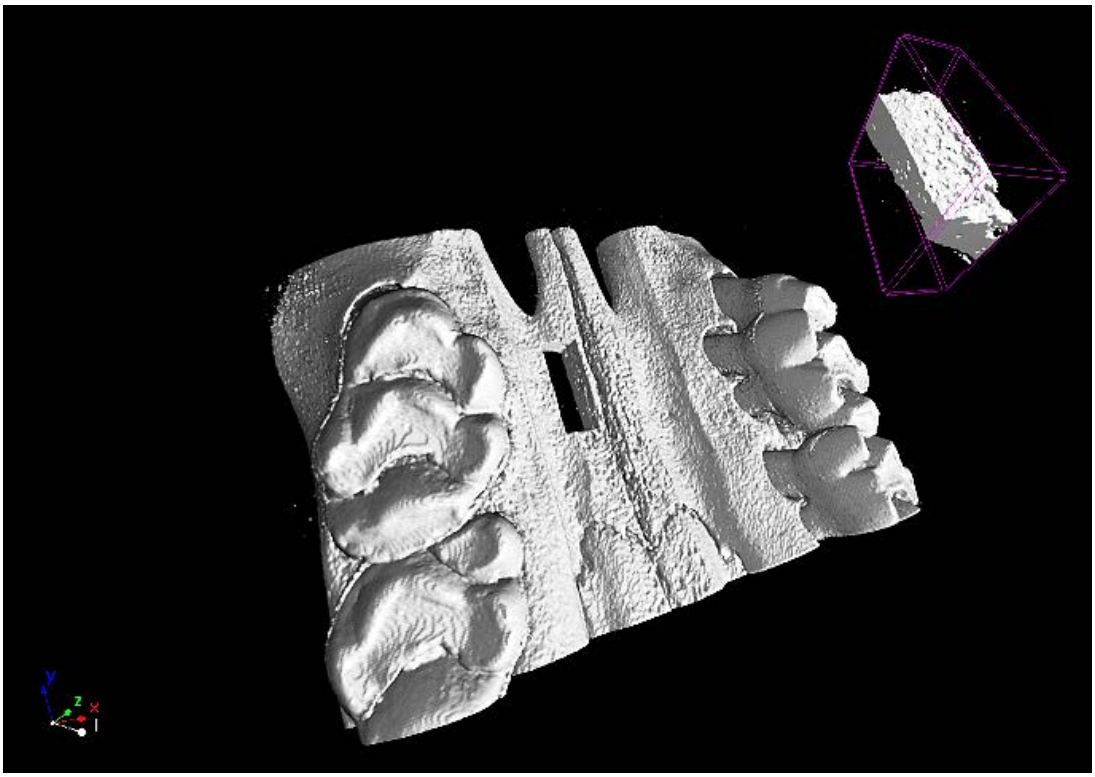


Figure 6. A reconstructed rat palate from a micro-CT scan, with a 1.5 mm by 0.5 mm area of palatal bone near the suture digitally sequestered for bone volume and greyscale analysis using Ctan software (v1.13.5.1; Skyscan).

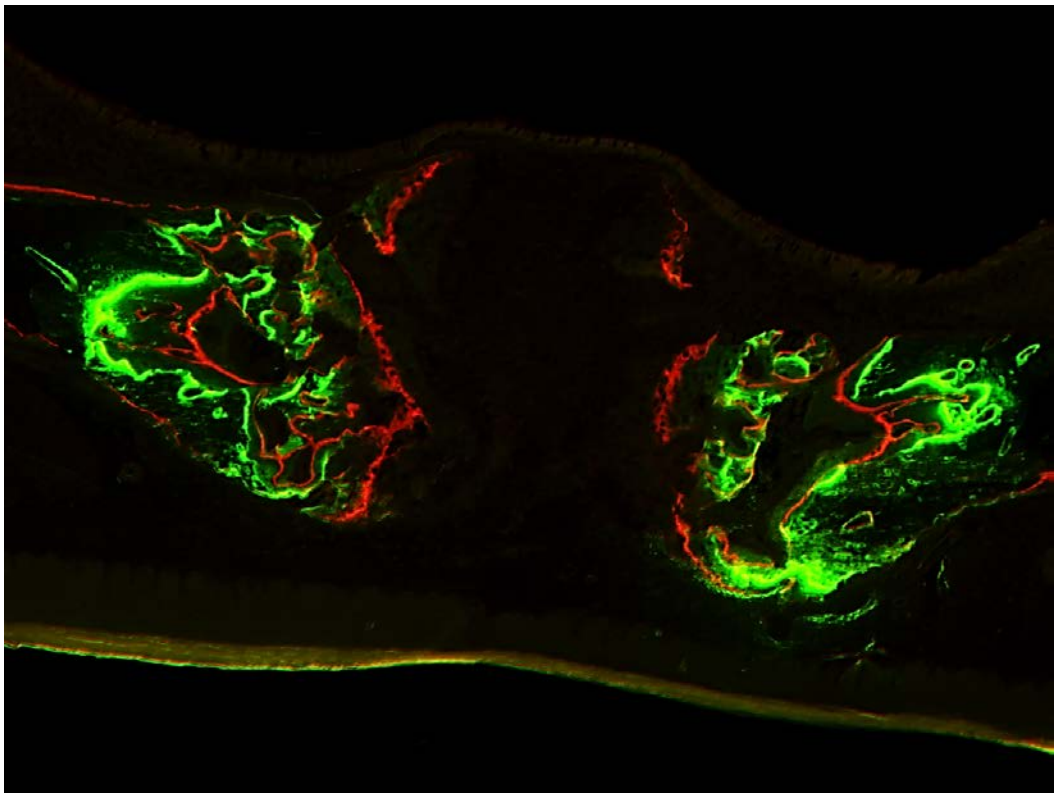


Figure 7. Epifluorescent micrograph of rat after maxillary expansion.

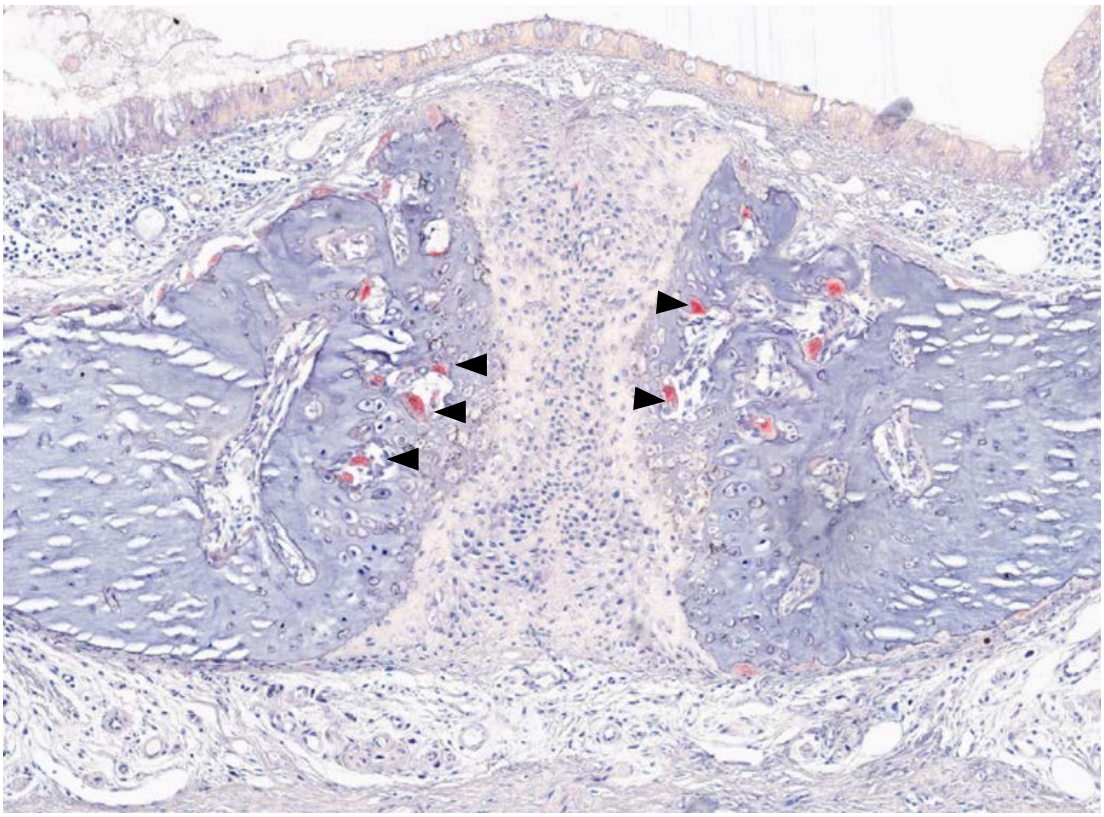
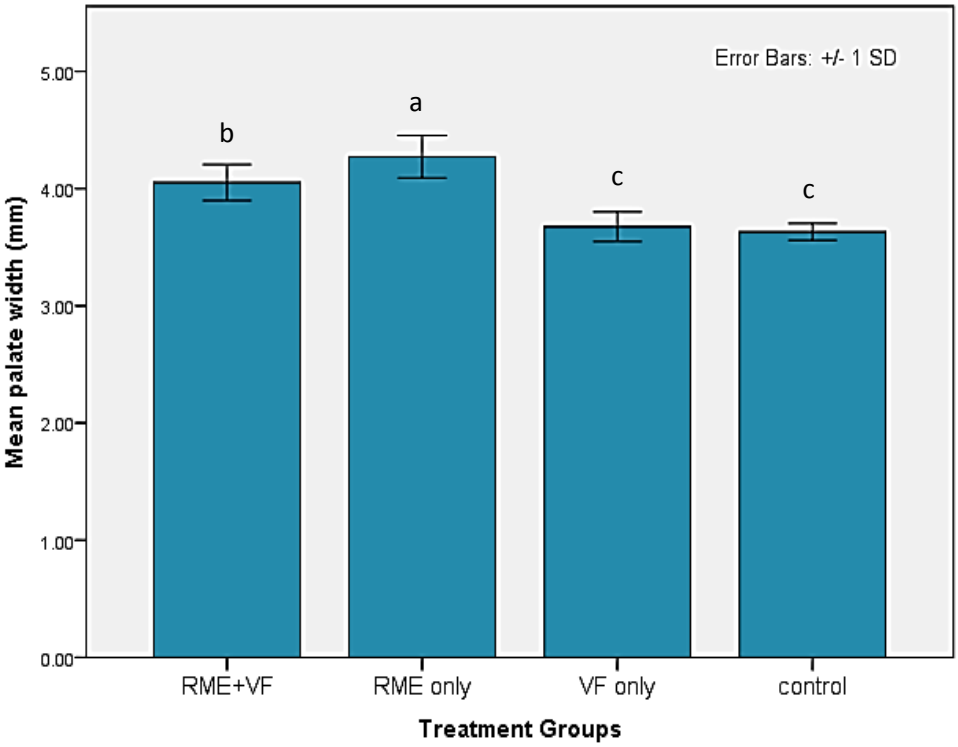
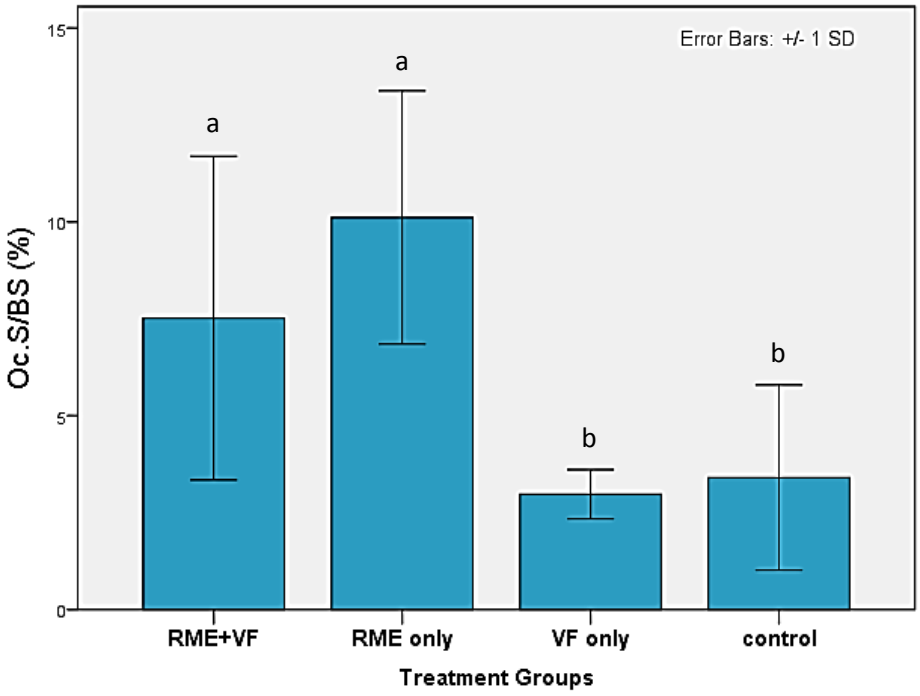


Figure 8. Tartrate-resistant acid phosphatase (TRAP) stained section of the palatal suture of a rat after undergoing expansion. TRAP positive cells in red (arrowheads).



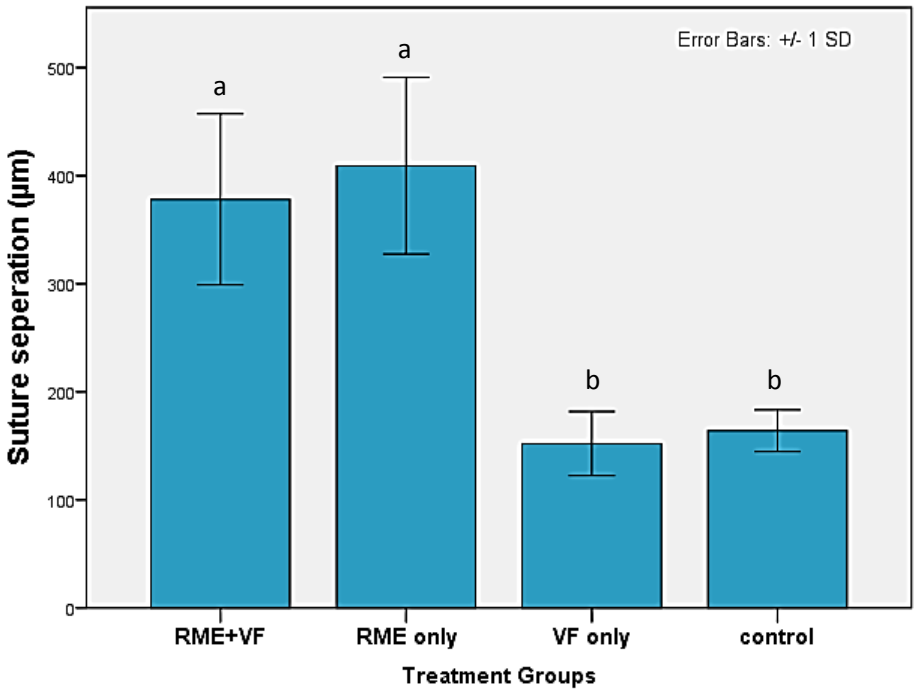
	RME+VF	RME only	VF only	control
Mean (SD)(mm)	4.05 (0.15) [b]	4.27 (0.17) [a]	3.67 (0.12) [c]	3.63 (0.07) [c]

Figure 9. Mean (SD) palatal widths after 2 weeks. [a, b, c] different letters denotes significance. ($p<0.05$)



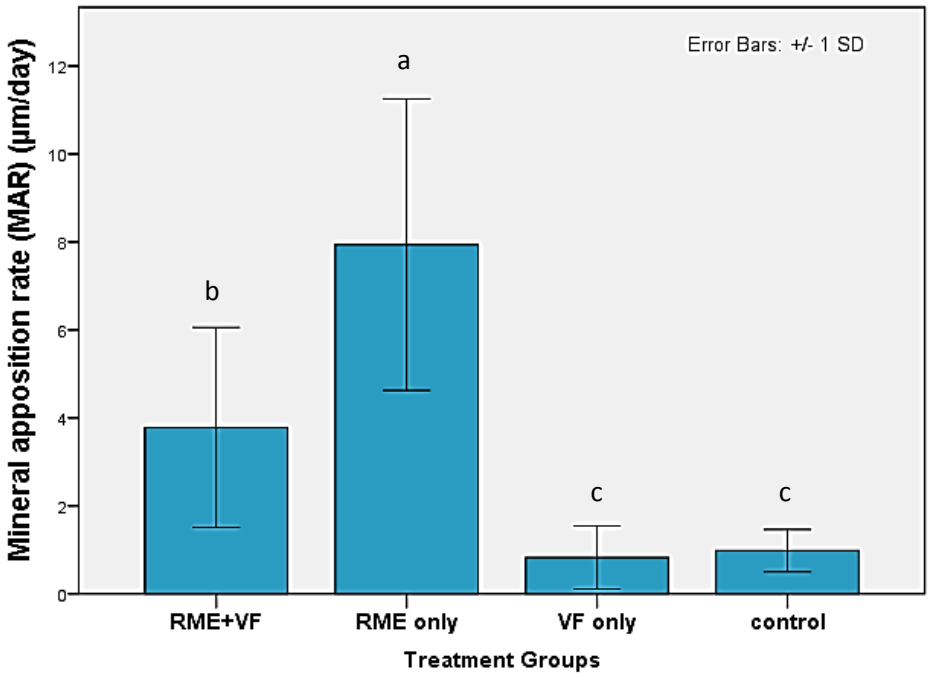
	RME+VF	RME only	VF only	control
Mean (SD)(%)	7.5 (4.1) [a]	10.11 (3.2) [a]	2.8 (0.5) [b]	3.6 (2.5) [b]

Figure 10. Mean (SD) of osteoclast surface/ bone surface ratios (Oc.S/ BS)(%) at the suture of the palatal bone after 2 weeks. [a, b] different letters denotes significance. ($p<0.05$)



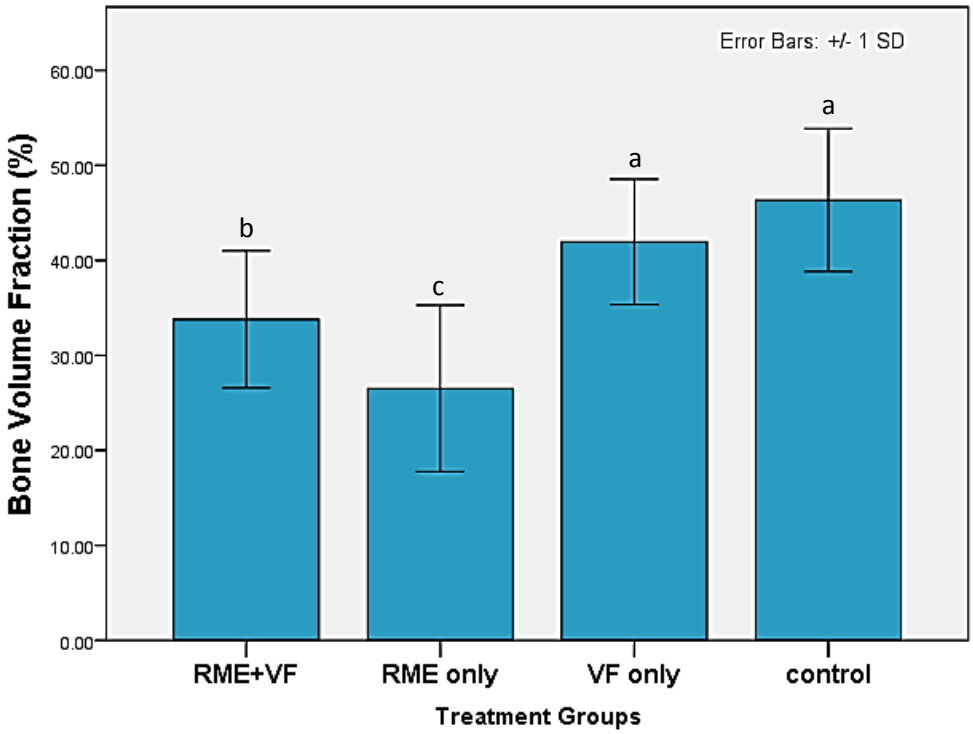
	RME+VF	RME only	VF only	control
Mean (SD)(µm)	378 (79)[a]	409 (81)[a]	152 (29)[b]	164 (19)[b]

Figure 11. Mean (SD) suture separation after 2 weeks. [a, b] *different letters denotes significance. ($p<0.05$)*



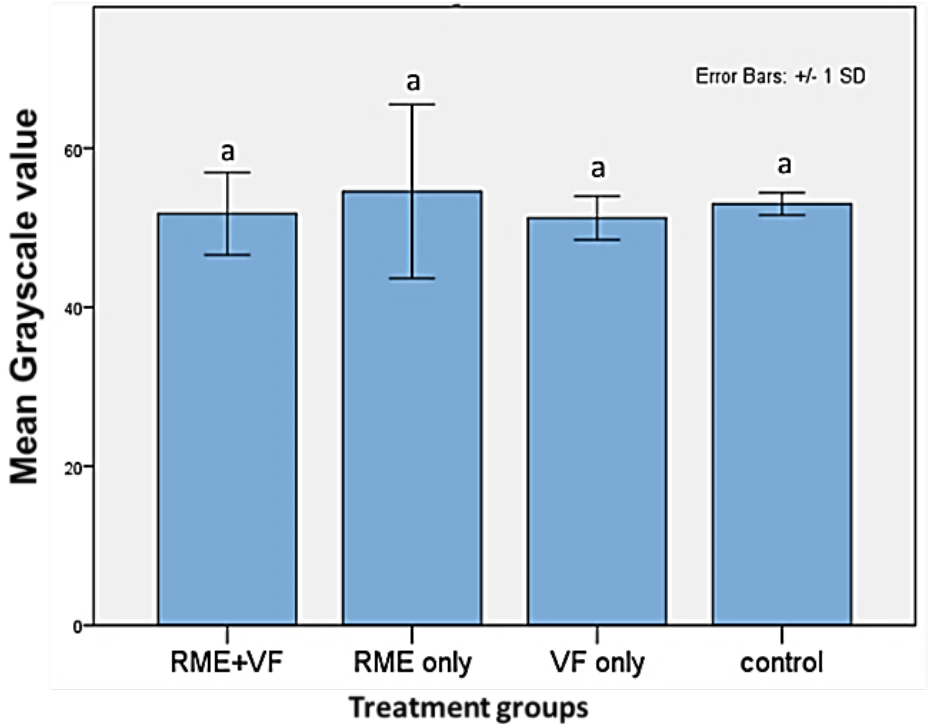
	RME+VF	RME only	VF only	control
Mean (SD)(µm/day)	3.8 (2.3) [b]	7.9 (3.3) [a]	0.90 (0.7) [c]	1.1 (0.4) [c]

Figure 12. Mean (SD) of the mineral apposition rates (MAR) at the suture of the palatal bone after 2 weeks. [a, b, c] different letters denotes significance. ($p<0.05$)



	RME+VF	RME only	VF only	control
Mean (SD)(%)	33.8 (7.2)[b]	26.5 (8.8) [c]	41.9 (6.6) [a]	46.3 (3.0) [a]

Figure 13. Mean (SD) of bone volume fractions of the palatal bone after 2 weeks. [a, b, c] different letters denotes significance. ($p<0.05$)



	RME+VF	RME only	VF only	control
Mean (SD)	51.8 (5.2) [a]	54.6 (11) [a]	51.2 (2.7) [a]	52.9 (1.4) [a]

Figure 14. Mean (SD) of greyscale values of the palatal bone after 2 weeks. [a] different letters denotes significance. ($p<0.05$)

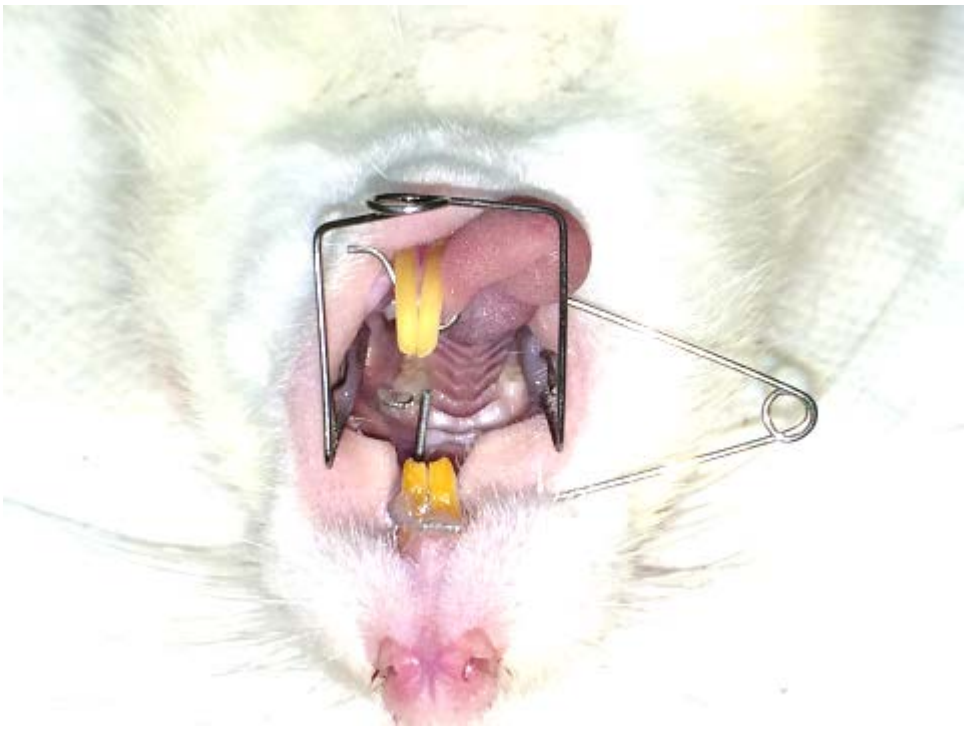


Figure 15. Orthodontic tooth movement (OTM) performed with bonded closed nickel titanium spring.

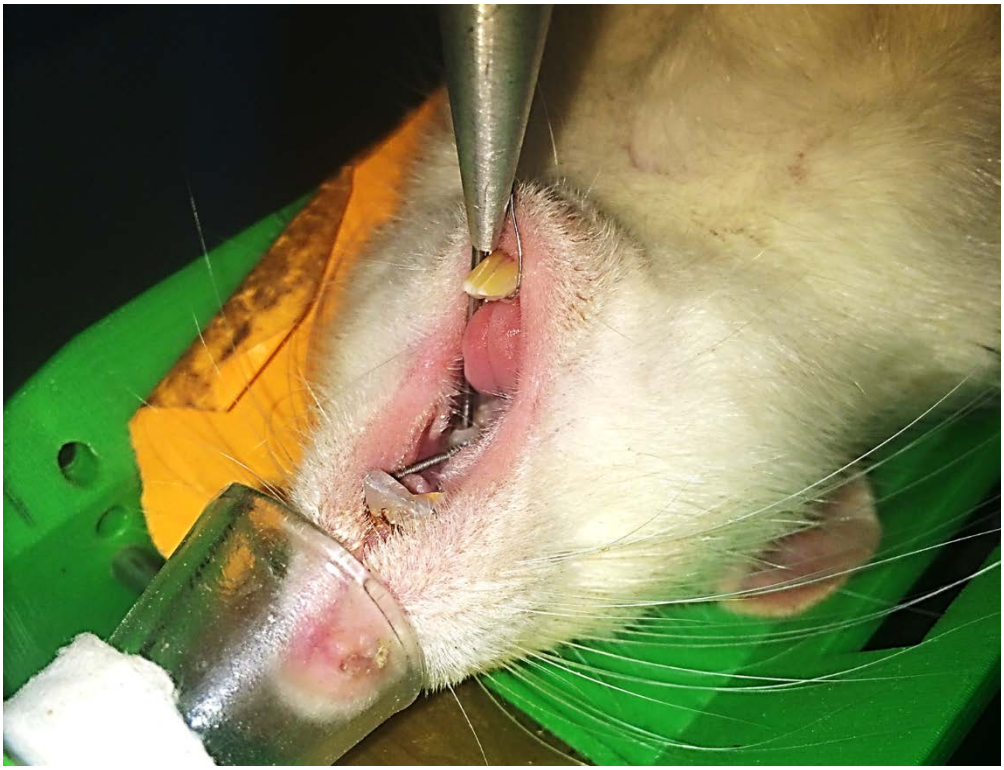
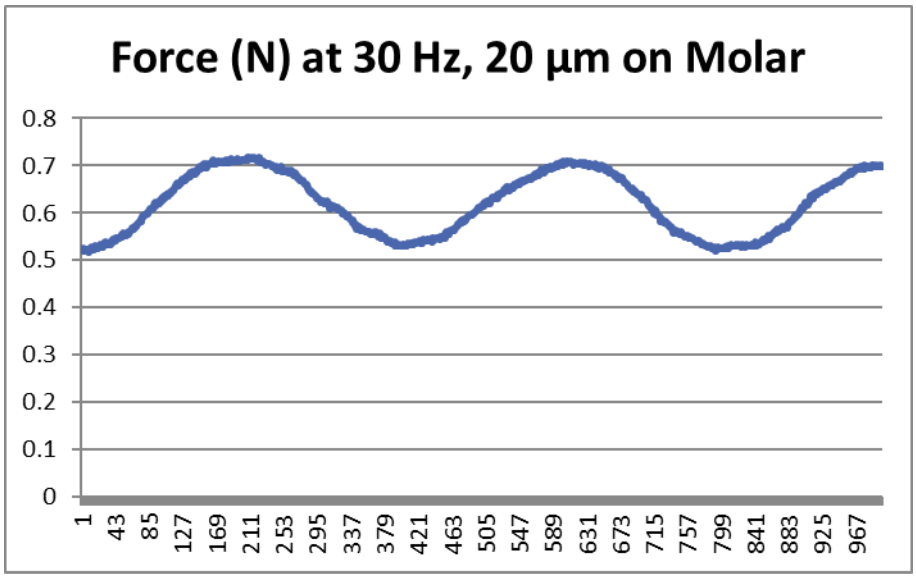


Figure 16. Intra-oral photograph showing the actuator tip delivering VF over the left maxillary first molar.



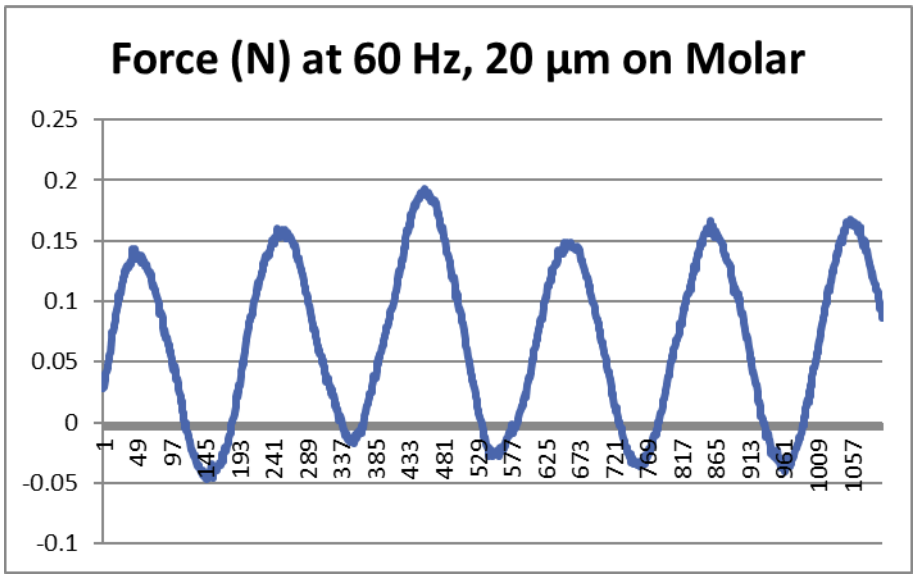


Figure 17. Typical transducer readings during VF treatments. Graph represents a 100 millisecond window, thus including six peaks of a 60 Hz waveform.

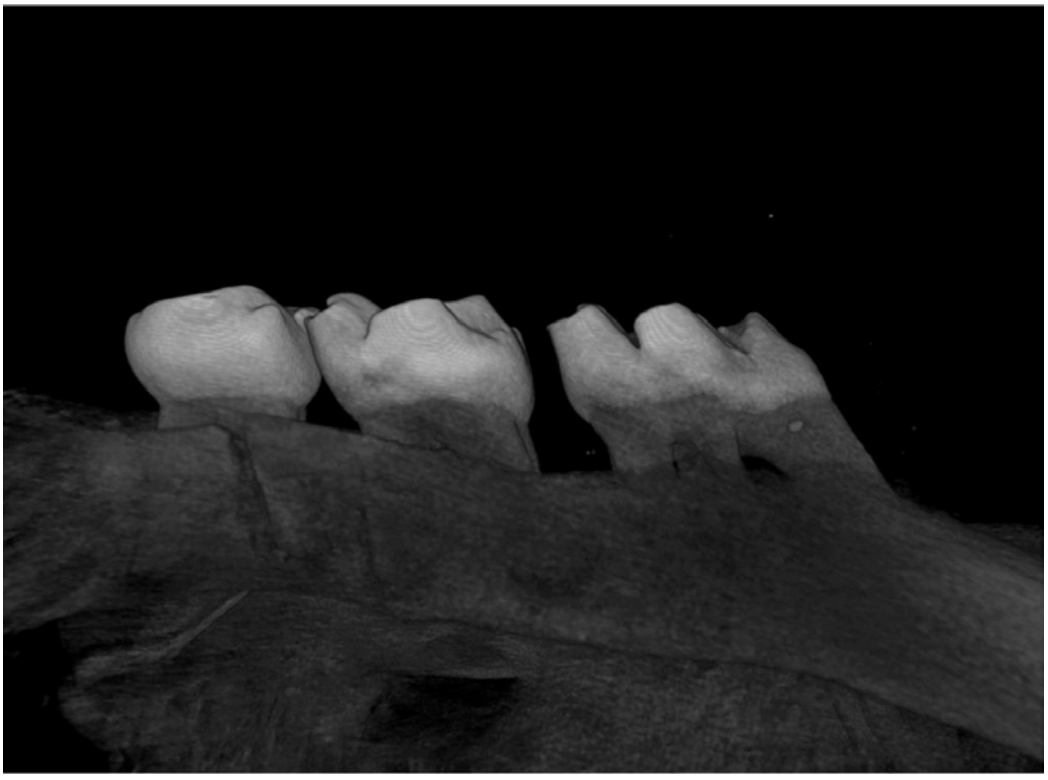


Figure 18. Micro-CT reconstructed image of a maxillary left molar after orthodontic tooth movement.

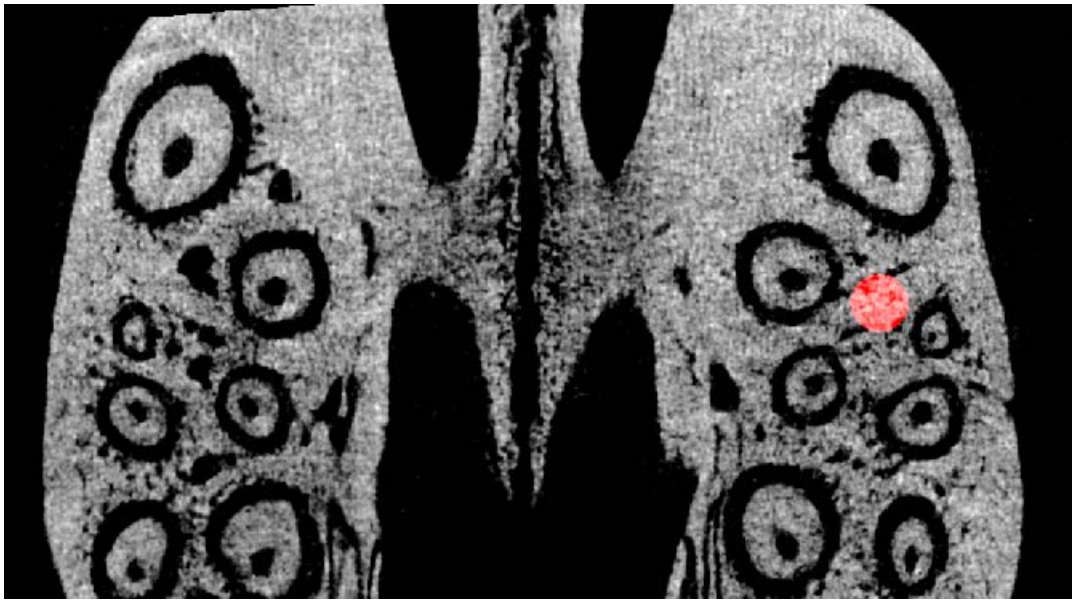


Figure 19. Micro-CT image slice showing the intra-radicular area of interest (ROI) used for bone analysis (highlighted in red).

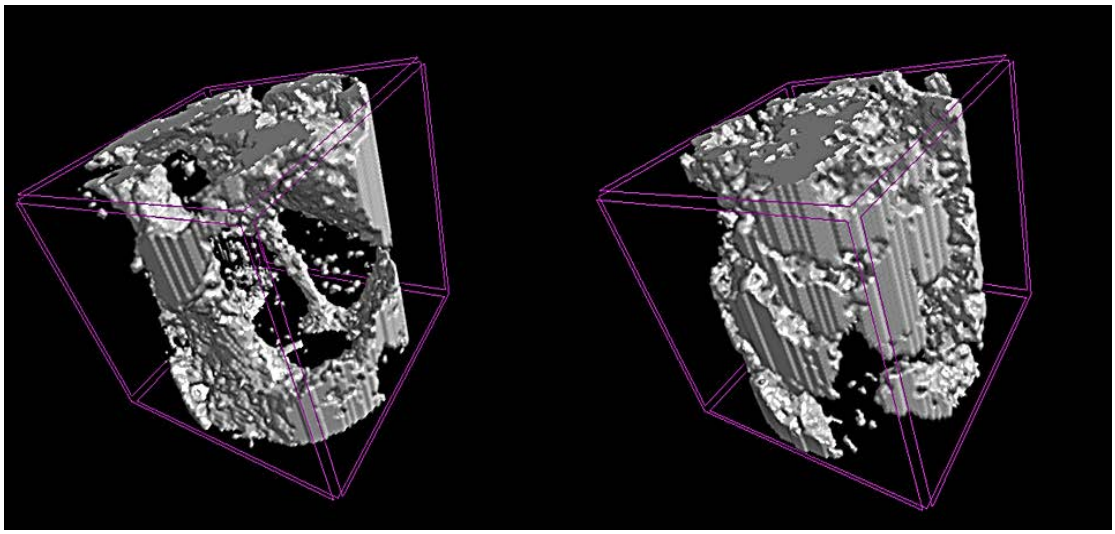
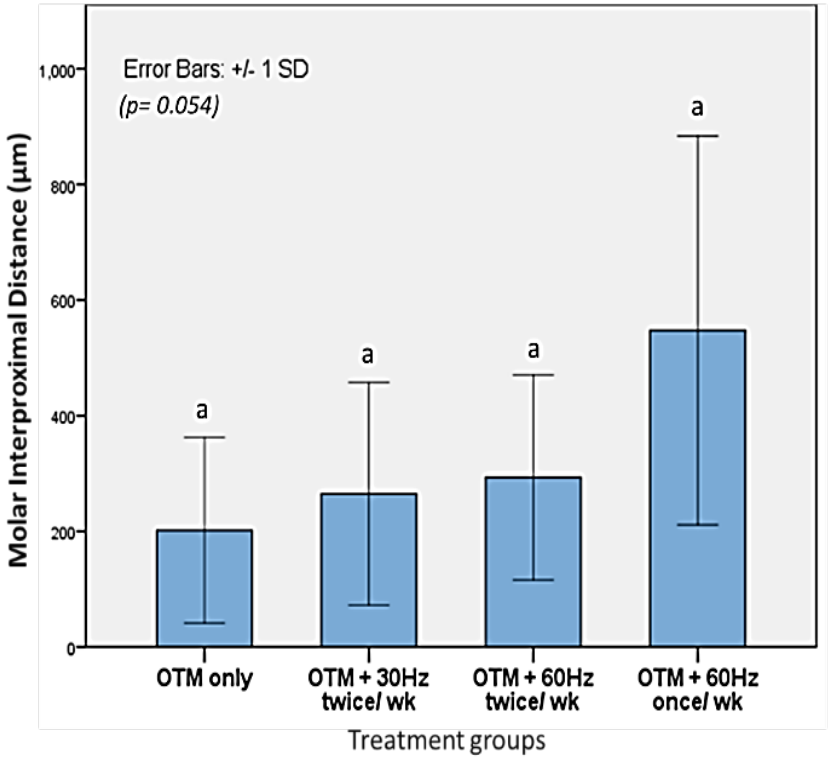
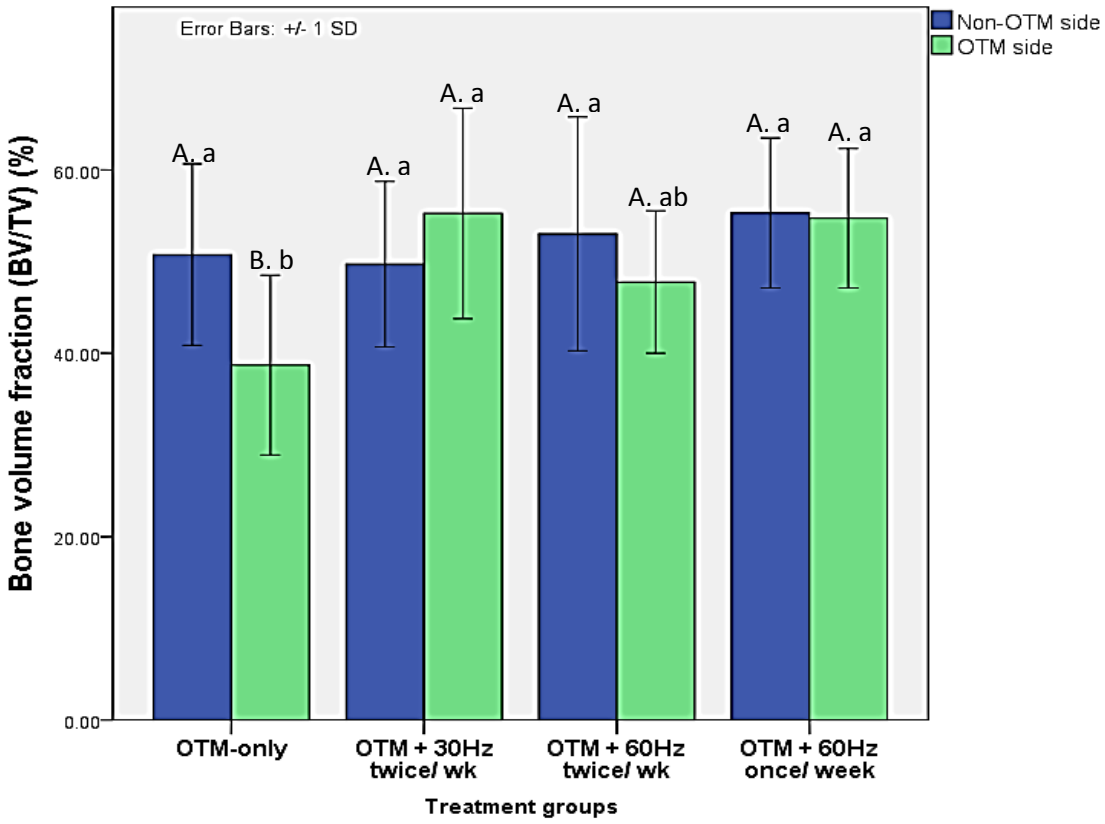


Figure 20. Micro-CT 3- dimensional reconstruction images showing the intra-radicular area of interest (ROI) used for bone analysis.



Group	OTM only	OTM + VF 30Hz twice/ week	OTM + VF 60Hz twice/ week	OTM + VF 60Hz once/ week
Mean separation (SD)	201.9 µm (160.22)	264.6 µm (192.2)	293.3 µm (176.9)	547.7 µm (336.1)

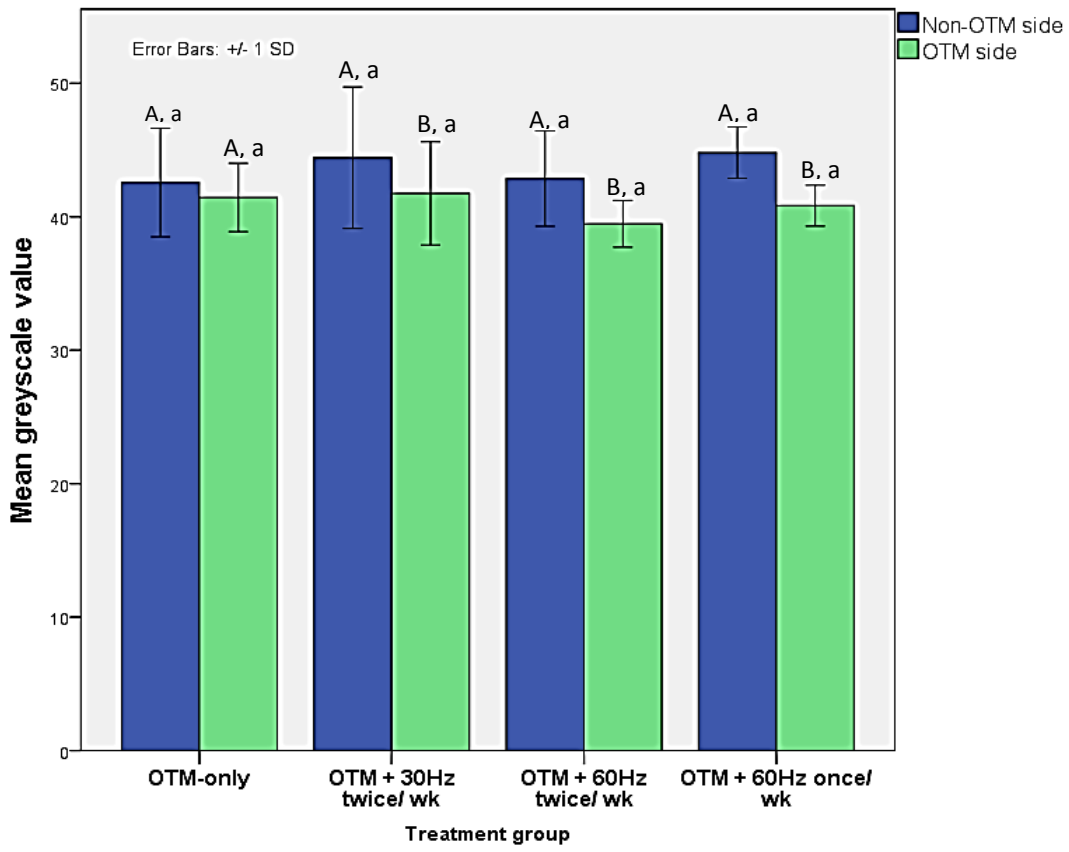
Figure 21. Mean (SD) 1st and 2nd interproximal distance after 4 weeks of OTM. [a] different letters denotes significance. ($p<0.05$)



Intra-radicular bone BV/TV % - Mean (SD)

Group	OTM only	OTM + VF 30Hz twice/ week	OTM + VF 60Hz twice/ week	OTM + VF 60Hz once/ week
non-OTM side	50.77(9.9) [A, a]	49.73 (9.0) [A, a]	53.03 (12.77) [A, a]	55.31(8.1) [A, a]
OTM-side	38.7(9.8) [B, b]	55.26 (11.5)[A, a]	47.78 (7.8) [A, ab]	54.74 (7.6) [A, a]

Figure 22. Mean (SD) of the bone volume fraction (BV/TV) of the Intra-radicular bone after 4 weeks of OTM. Different upper-case letters denotes significance within the same group. ($p<0.05$) Different lower-case letters denotes significance between groups. ($p<0.05$)



Intra-radicular bone mean grayscale values – Mean (SD)

Group	OTM only	OTM + VF 30Hz twice/ week	OTM + VF 60Hz twice/ week	OTM + VF 60Hz once/ week
non-OTM side	42.56(4.1) [A, a]	44.43 (5.3) [A, a]	42.86 (3.6) [A, a]	44.8(1.9) [A, a]
OTM-side	41.44(2.6) [A, a]	41.75 (3.9) [B, a]	39.47 (1.8) [B, a]	40.83 (1.5) [B, a]

Figure 23. Mean (SD) of the mean grayscale values of the intra-radicular bone after 4 weeks of OTM. Different upper-case letters denotes significance within the same group. ($p<0.05$) Different lower-case letters denotes significance between groups. ($p<0.05$)

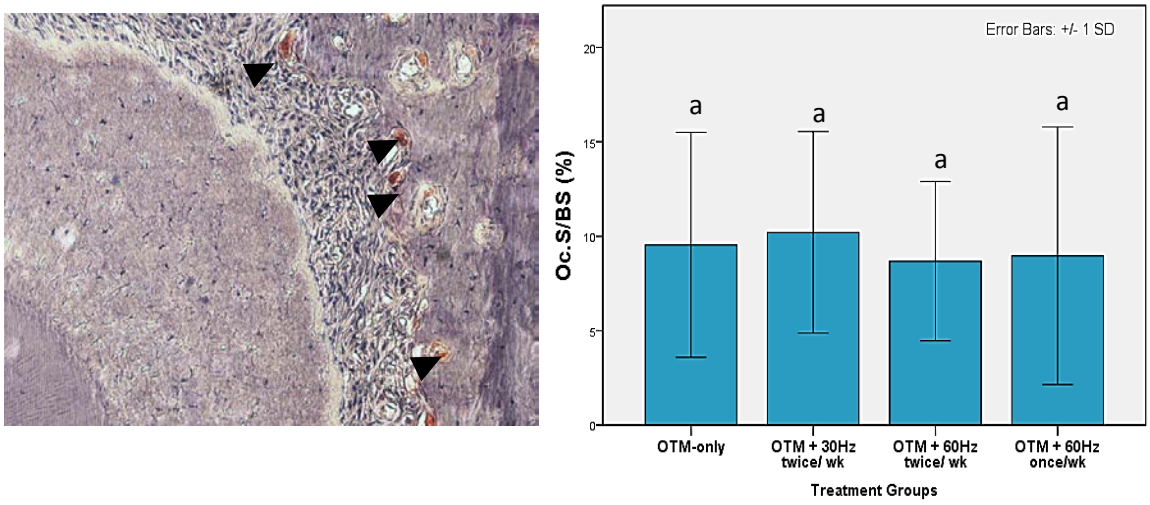


Figure 24. Tartrate-resistant acid phosphatase (TRAP) stained section of alveolar bone anterior to the mesial root of the maxillary 1st molar after OTM. TRAP positive cells in red (arrowheads). [a] No statistical significance at ($p \leq 0.05$)

Figure 25: Positioning of the maxillary expander against the palate of the mouse.

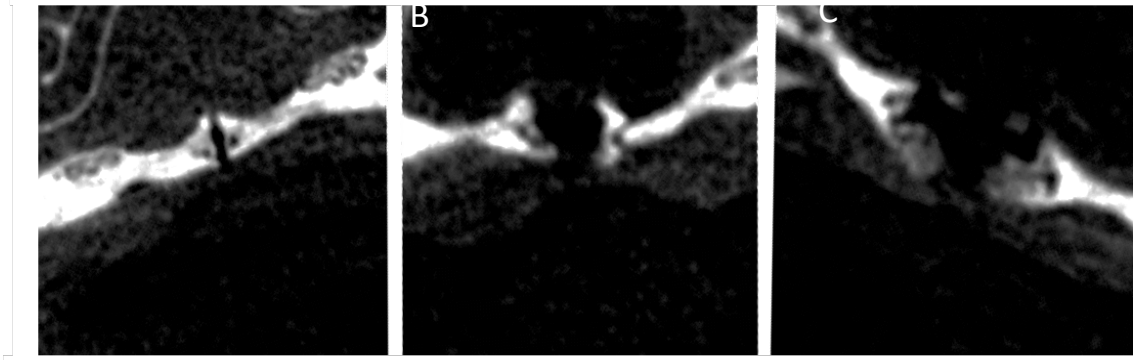


Figure 26. Verification of suture separation using μCT of the controls (A), 10g (B) and 20g groups (C).

Group	Suture width (SD) (µm)*	BV/TV (SD) (%)*	MAR (SD) (µm/day)*	Periostin expression#
Control	92(8) [c]	89.4(2.3) [b]	0.9(0.77) [c]	(+) [c]
10g	247(116) [b]	85.7(5.5) [b]	2.7(1.2) [b]	(++) [b]
20g	413(98) [a]	77.1(8.5) [a]	4.5(2.2) [a]	(+++) [a]

Table 1. Summary of results. (Different letters denotes significant differences)

* One-way ANOVA ($p<0.05$)

Mantel -Haenszel Chi-square ($p<0.05$)

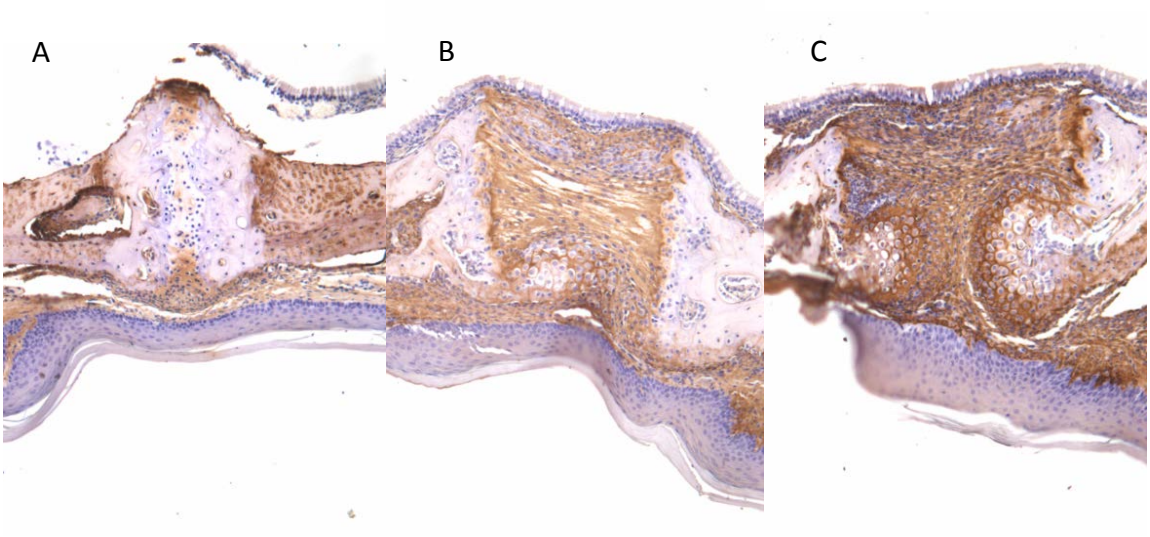


Figure 27. Microphotographs of iImmunohistochemistry staining for periostin in representative sections of control (A) 10g (B) and 20g groups (C). Both experimental groups showed increased expression of POSTN on the surfaces of the suture bone and along the bundles of connective fibers within the suture area (B, C). Positive staining was largely confined to oral and nasal borders of the sutures in the controls (A).

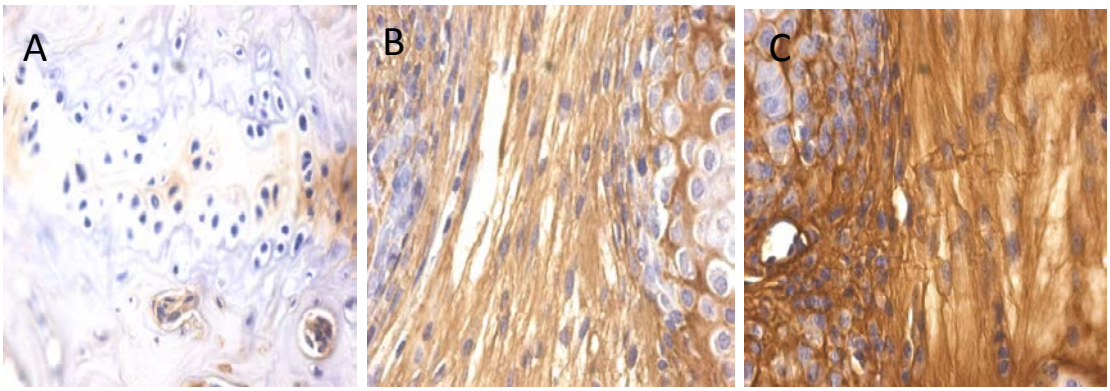


Figure 28. 40x Magnification of suture area showing the pattern and intensity of the positive staining for POSTN in representative sections of 20g (A), 10g (B) and control groups (C).

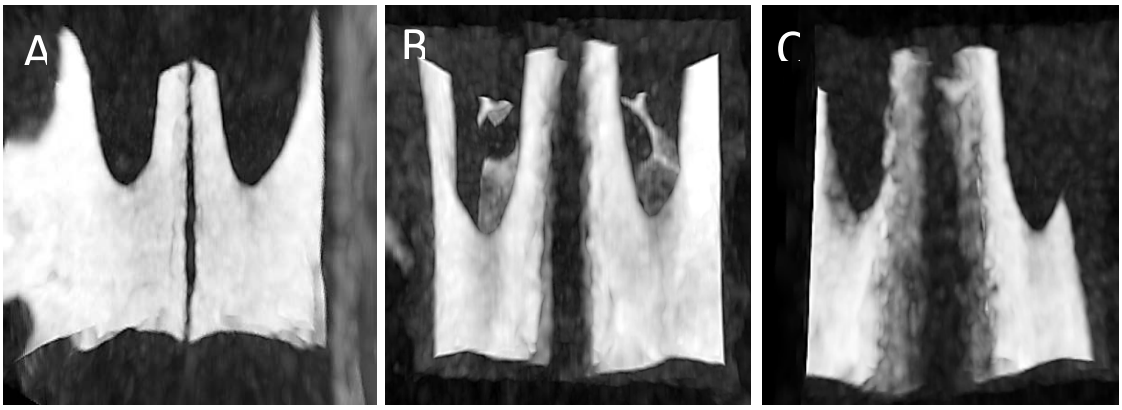


Figure 29. Three dimensional reconstruction of the suture area. High degree of bone remodeling is evident in the 20g group resulting in lower bone volume fractions (C) compared to the 10g group (B) and control (A).

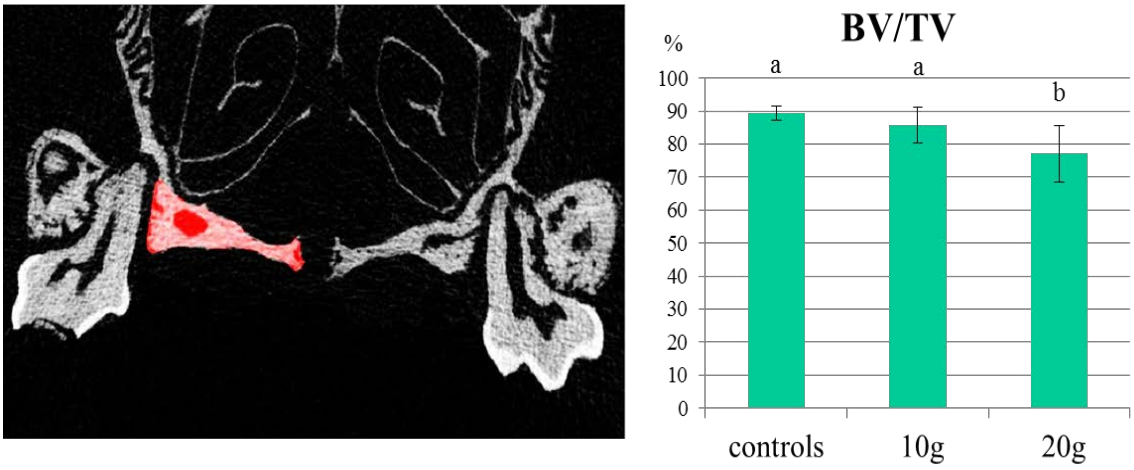


Figure 30. Bone volume to total volume ratios (BV/TV)(%). The bone volume density was reduced in the 20g group by 12.1% when compared to the baseline (different letters denotes significant differences, $p < 0.05$).

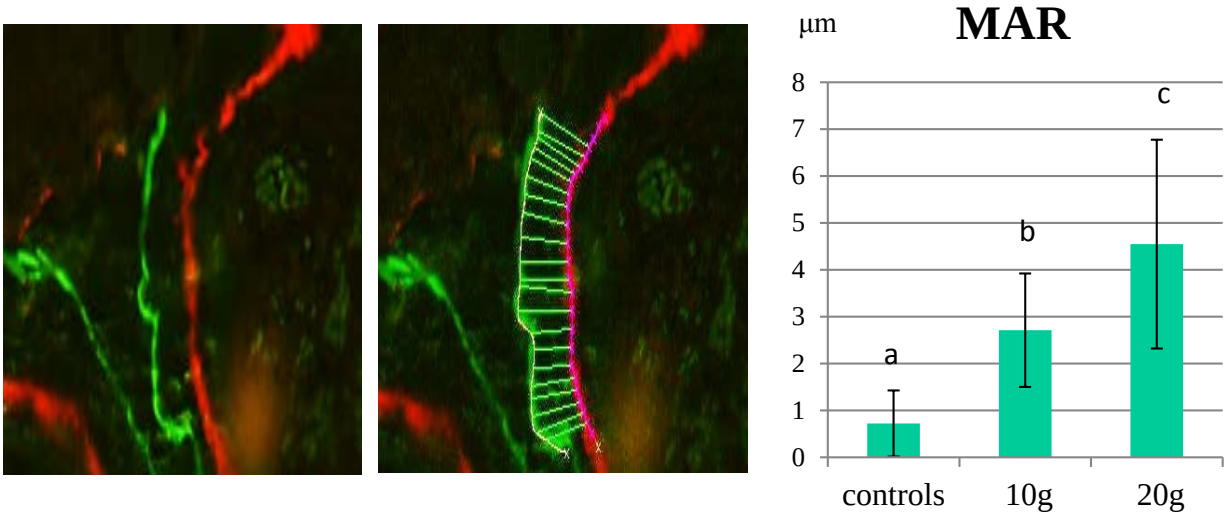


Figure 31. Bioquant Osteo© software was used to measure mineral apposition rates. The 20g group had an average mineral opposition rate of 4.5 ± 2.2 µm of bone per day, the 10g group had an average of 2.7 ± 1.2 µm and the controls laid an average of less than 1 ± 0.77 µm/ day (different letters denotes significant differences, $p < 0.05$).

REFERENCES

Agou, S., Locker, D., Streiner, D. L., & Tompson, B. (2008). Impact of self-esteem on the oral-health-related quality of life of children with malocclusion. *Am J Orthod Dentofacial Orthop, 134*(4), 484-489. doi: 10.1016/j.ajodo.2006.11.021

Alikhani, M., Khoo, E., Alyami, B., Raptis, M., Salgueiro, J. M., Oliveira, S. M., . . . Teixeira, C. C. (2012). Osteogenic effect of high-frequency acceleration on alveolar bone. *J Dent Res, 91*(4), 413-419. doi: 10.1177/0022034512438590

Andiappan, M., Gao, W., Bernabe, E., Kandala, N. B., & Donaldson, A. N. (2014). Malocclusion, orthodontic treatment, and the Oral Health Impact Profile (OHIP-14) Systematic review and meta-analysis. *Angle Orthod*. doi: 10.2319/051414-348.1

Badran, S. A., Sabrah, A. H., Hadidi, S. A., & Al-Khateeb, S. (2014). Effect of socioeconomic status on normative and perceived orthodontic treatment need. *Angle Orthod, 84*(4), 588-593. doi: 10.2319/062913-482.1

Barbagallo, L. J., Jones, A. S., Petocz, P., & Darendeliler, M. A. (2008). Physical properties of root cementum: Part 10. Comparison of the effects of invisible removable thermoplastic appliances with light and heavy orthodontic forces on premolar cementum. A microcomputed-tomography study. *Am J Orthod Dentofacial Orthop, 133*(2), 218-227. doi: 10.1016/j.ajodo.2006.01.043

Barbosa Tde, S., Tureli, M. C., Nobre-dos-Santos, M., Puppin-Rontani, R. M., & Gaviao, M. B. (2013). The relationship between oral conditions, masticatory performance and oral health-related quality of life in children. *Arch Oral Biol, 58*(9), 1070-1077. doi: 10.1016/j.archoralbio.2013.01.012

Bishara, S. E., & Staley, R. N. (1987). Maxillary expansion: clinical implications. *Am J Orthod Dentofacial Orthop, 91*(1), 3-14.

Bonnet, N., Standley, K. N., Bianchi, E. N., Stadelmann, V., Foti, M., Conway, S. J., & Ferrari, S. L. (2009). The matricellular protein periostin is required for sost inhibition and the anabolic response to mechanical loading and physical activity. *J Biol Chem, 284*(51), 35939-35950. doi: 10.1074/jbc.M109.060335

Borzabadi-Farahani, A., Eslamipour, F., & Asgari, I. (2011). Association between orthodontic treatment need and caries experience. *Acta Odontol Scand, 69*(1), 2-11. doi: 10.3109/00016357.2010.516732

Bouxsein, M. L., Boyd, S. K., Christiansen, B. A., Guldberg, R. E., Jepsen, K. J., & Muller, R. (2010). Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J Bone Miner Res, 25*(7), 1468-1486. doi: 10.1002/jbmr.141

Brunelle, J. A., Bhat, M., & Lipton, J. A. (1996). Prevalence and distribution of selected occlusal characteristics in the US population, 1988-1991. *J Dent Res, 75 Spec No*, 706-713.

Butcher, J. T., Norris, R. A., Hoffman, S., Mjaatvedt, C. H., & Markwald, R. R. (2007). Periostin promotes atrioventricular mesenchyme matrix invasion and remodeling mediated by integrin signaling through Rho/PI 3-kinase. *Dev Biol, 302*(1), 256-266. doi: 10.1016/j.ydbio.2006.09.048

Cepera, F., Torres, F. C., Scanavini, M. A., Paranhos, L. R., Capelozza Filho, L., Cardoso, M. A., . . . Siqueira, D. F. (2012). Effect of a low-level laser on bone regeneration after rapid maxillary expansion. *Am J Orthod Dentofacial Orthop, 141*(4), 444-450. doi: 10.1016/j.ajodo.2011.10.023

Conway, S. J., Doetschman, T., & Azhar, M. (2011). The inter-relationship of periostin, TGF beta, and BMP in heart valve development and valvular heart diseases. *ScientificWorldJournal, 11*, 1509-1524. doi: 10.1100/tsw.2011.132

Cowan, C. M., Soo, C., Ting, K., & Wu, B. (2005). Evolving concepts in bone tissue engineering. *Curr Top Dev Biol, 66*, 239-285. doi: 10.1016/S0070-2153(05)66008-5

Cullen, D. M., Smith, R. T., & Akhter, M. P. (2001). Bone-loading response varies with strain magnitude and cycle number. *J Appl Physiol (1985), 91*(5), 1971-1976.

da Silva, A. P., Petri, A. D., Crippa, G. E., Stuani, A. S., Stuani, A. S., Rosa, A. L., & Stuani, M. B. (2012). Effect of low-level laser therapy after rapid maxillary expansion on proliferation and differentiation of osteoblastic cells. *Lasers Med Sci, 27*(4), 777-783. doi: 10.1007/s10103-011-0968-0

de Oliveira, M. L., Bergamaschi, C. T., Silva, O. L., Nonaka, K. O., Wang, C. C., Carvalho, A. B., . . . Lazaretti-Castro, M. (2010). Mechanical vibration preserves bone structure in rats treated with glucocorticoids. *Bone, 46*(6), 1516-1521. doi: 10.1016/j.bone.2010.02.009

Duncan, R. L., & Turner, C. H. (1995). Mechanotransduction and the functional response of bone to mechanical strain. *Calcif Tissue Int, 57*(5), 344-358.

Ekizer, A., Yalvac, M. E., Uysal, T., Sonmez, M. F., & Sahin, F. (2014). Bone marrow mesenchymal stem cells enhance bone formation in orthodontically expanded maxillae in rats. *Angle Orthod*. doi: 10.2319/031114-177.1

Ekstrom, C., Henrikson, C. O., & Jensen, R. (1977). Mineralization in the midpalatal suture after orthodontic expansion. *Am J Orthod, 71*(4), 449-455.

Fisher, M. A., Wenger, R. M., & Hans, M. G. (2010). Pretreatment characteristics associated with orthodontic treatment duration. *Am J Orthod Dentofacial Orthop, 137*(2), 178-186. doi: 10.1016/j.ajodo.2008.09.028

Gilsanz, V., Wren, T. A., Sanchez, M., Dorey, F., Judex, S., & Rubin, C. (2006). Low-level, high-frequency mechanical signals enhance musculoskeletal development of young women with low BMD. *J Bone Miner Res, 21*(9), 1464-1474. doi: 10.1359/jbmr.060612

Gonzales, C., Hotokezaka, H., Yoshimatsu, M., Yozgatian, J. H., Darendeliler, M. A., & Yoshida, N. (2008). Force magnitude and duration effects on amount of tooth movement and root resorption in the rat molar. *Angle Orthod, 78*(3), 502-509. doi: 10.2319/052007-240.1

Habersack, K., Karoglan, A., Sommer, B., & Benner, K. U. (2007). High-resolution multislice computerized tomography with multiplanar and 3-dimensional reformation imaging in rapid palatal expansion. *Am J Orthod Dentofacial Orthop, 131*(6), 776-781. doi: 10.1016/j.ajodo.2005.09.030

Henneman, S., Von den Hoff, J. W., & Maltha, J. C. (2008). Mechanobiology of tooth movement. *Eur J Orthod, 30*(3), 299-306. doi: 10.1093/ejo/cjn020

Hicks, E. P. (1978). Slow maxillary expansion. A clinical study of the skeletal versus dental response to low-magnitude force. *Am J Orthod, 73*(2), 121-141.

Horiuchi, K., Amizuka, N., Takeshita, S., Takamatsu, H., Katsuura, M., Ozawa, H., . . . Kudo, A. (1999). Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. *J Bone Miner Res, 14*(7), 1239-1249. doi: 10.1359/jbmr.1999.14.7.1239

Jarvinen, S., Widstrom, E., & Raitio, M. (2004). Factors affecting the duration of orthodontic treatment in children. A retrospective study. *Swed Dent J, 28*(2), 93-100.

Kalajzic, Z., Peluso, E. B., Utreja, A., Dyment, N., Nihara, J., Xu, M., . . . Wadhwa, S. (2014). Effect of cyclical forces on the periodontal ligament and alveolar bone remodeling during orthodontic tooth movement. *Angle Orthod, 84*(2), 297-303. doi: 10.2319/032213-234.1

Karadeniz, E. I., Gonzales, C., Nebioglu-Dalci, O., Dwarte, D., Turk, T., Isci, D., . . . Darendeliler, M. A. (2011). Physical properties of root cementum: part 20. Effect of fluoride on orthodontically induced root resorption with light and heavy orthodontic forces for 4 weeks: a microcomputed tomography study. *Am J Orthod Dentofacial Orthop, 140*(5), e199-210. doi: 10.1016/j.ajodo.2011.05.017

Kashima, T. G., Nishiyama, T., Shimazu, K., Shimazaki, M., Kii, I., Grigoriadis, A. E., . . . Kudo, A. (2009). Periostin, a novel marker of intramembranous ossification, is expressed in fibrous dysplasia and in c-Fos-overexpressing bone lesions. *Hum Pathol, 40*(2), 226-237. doi: 10.1016/j.humpath.2008.07.008

Kasturi, G., & Adler, R. A. (2011). Mechanical means to improve bone strength: ultrasound and vibration. *Curr Rheumatol Rep, 13*(3), 251-256. doi: 10.1007/s11926-011-0177-7

Katebi, N., Kolpakova-Hart, E., Lin, C. Y., & Olsen, B. R. (2012). The mouse palate and its cellular responses to midpalatal suture expansion forces. *Orthod Craniofac Res, 15*(3), 148-158. doi: 10.1111/j.1601-6343.2012.01547.x

Keim, R. G., Gottlieb, E. L., Nelson, A. H., & Vogels, D. S., 3rd. (2008). 2008 JCO study of orthodontic diagnosis and treatment procedures, part 1: results and trends. *J Clin Orthod, 42*(11), 625-640.

Kohno, T., Matsumoto, Y., Kanno, Z., Warita, H., & Soma, K. (2002). Experimental tooth movement under light orthodontic forces: rates of tooth movement and changes of the periodontium. *J Orthod, 29*(2), 129-135.

Kono, T., Ayukawa, Y., Moriyama, Y., Kurata, K., Takamatsu, H., & Koyano, K. (2012). The effect of low-magnitude, high-frequency vibration stimuli on the bone healing of rat incisor extraction socket. *J Biomech Eng, 134*(9), 091001. doi: 10.1115/1.4007247

Kopher, R. A., & Mao, J. J. (2003). Suture growth modulated by the oscillatory component of micromechanical strain. *J Bone Miner Res, 18*(3), 521-528. doi: 10.1359/jbmr.2003.18.3.521

Kruzynska-Frejtag, A., Machnicki, M., Rogers, R., Markwald, R. R., & Conway, S. J. (2001). Periostin (an osteoblast-specific factor) is expressed within the embryonic mouse heart during valve formation. *Mech Dev, 103*(1-2), 183-188.

Lau, E., Al-Dujaili, S., Guenther, A., Liu, D., Wang, L., & You, L. (2010). Effect of low-magnitude, high-frequency vibration on osteocytes in the regulation of osteoclasts. *Bone, 46*(6), 1508-1515. doi: 10.1016/j.bone.2010.02.031

Lau, K. H., Kapur, S., Kesavan, C., & Baylink, D. J. (2006). Up-regulation of the Wnt, estrogen receptor, insulin-like growth factor-I, and bone morphogenetic protein pathways in C57BL/6J osteoblasts as opposed to C3H/HeJ osteoblasts in part contributes to the differential anabolic response to fluid shear. *J Biol Chem, 281*(14), 9576-9588. doi: 10.1074/jbc.M509205200

Liu, A. Y., Zheng, H., & Ouyang, G. (2014). Periostin, a multifunctional matricellular protein in inflammatory and tumor microenvironments. *Matrix Biol, 37C*, 150-156. doi: 10.1016/j.matbio.2014.04.007

Liu, S. S., Kyung, H. M., & Buschang, P. H. (2010). Continuous forces are more effective than intermittent forces in expanding sutures. *Eur J Orthod, 32*(4), 371-380. doi: 10.1093/ejo/cjp103

Liu, S. S., Opperman, L. A., Kyung, H. M., & Buschang, P. H. (2011). Is there an optimal force level for sutural expansion? *Am J Orthod Dentofacial Orthop, 139*(4), 446-455. doi: 10.1016/j.ajodo.2009.03.056

Liu, S. S., Xu, H., Sun, J., Kontogiorgos, E., Whittington, P. R., Misner, K. G., . . . Opperman, L. A. (2013). Recombinant human bone morphogenetic protein-2 stimulates bone formation during interfrontal suture expansion in rabbits. *Am J Orthod Dentofacial Orthop, 144*(2), 210-217. doi: 10.1016/j.ajodo.2013.03.017

Lv, S., Liu, H., Cui, J., Hasegawa, T., Hongo, H., Feng, W., . . . Li, M. (2014). Histochemical examination of cathepsin K, MMP1 and MMP2 in compressed periodontal ligament during orthodontic tooth movement in periostin deficient mice. *J Mol Histol, 45*(3), 303-309. doi: 10.1007/s10735-013-9548-x

Madan, M. S., Liu, Z. J., Gu, G. M., & King, G. J. (2007). Effects of human relaxin on orthodontic tooth movement and periodontal ligaments in rats. *Am J Orthod Dentofacial Orthop, 131*(1), 8 e1-10. doi: 10.1016/j.ajodo.2006.06.014

Magalhaes, I. B., Pereira, L. J., Marques, L. S., & Gameiro, G. H. (2010). The influence of malocclusion on masticatory performance. A systematic review. *Angle Orthod, 80*(5), 981-987. doi: 10.2319/011910-33.1

Martins-Junior, P. A., Marques, L. S., & Ramos-Jorge, M. L. (2012). Malocclusion: social, functional and emotional influence on children. *J Clin Pediatr Dent, 37*(1), 103-108.

Masood, Y., Masood, M., Zainul, N. N., Araby, N. B., Hussain, S. F., & Newton, T. (2013). Impact of malocclusion on oral health related quality of life in young people. *Health Qual Life Outcomes, 11*, 25. doi: 10.1186/1477-7525-11-25

Mavreas, D., & Athanasiou, A. E. (2008). Factors affecting the duration of orthodontic treatment: a systematic review. *Eur J Orthod, 30*(4), 386-395. doi: 10.1093/ejo/cjn018

McBride, S. H., & Silva, M. J. (2012). Adaptive and Injury Response of Bone to Mechanical Loading. *Bonekey Osteovision, 1*. doi: 10.1038/bonekey.2012.192

McComb, J. L. (1994). Orthodontic treatment and isolated gingival recession: a review. *Br J Orthod, 21*(2), 151-159.

Merle, B., Bouet, G., Rousseau, J. C., Bertholon, C., & Garnero, P. (2014). Periostin and transforming growth factor beta-induced protein (TGFbetaIp) are both expressed by osteoblasts and osteoclasts. *Cell Biol Int, 38*(3), 398-404. doi: 10.1002/cbin.10219

Merle, B., & Garnero, P. (2012). The multiple facets of periostin in bone metabolism. *Osteoporos Int, 23*(4), 1199-1212. doi: 10.1007/s00198-011-1892-7

Mizrahi, E. (1982). Enamel demineralization following orthodontic treatment. *Am J Orthod, 82*(1), 62-67.

Nimeri, G., Kau, C. H., Abou-Kheir, N. S., & Corona, R. (2013). Acceleration of tooth movement during orthodontic treatment--a frontier in orthodontics. *Prog Orthod, 14*, 42. doi: 10.1186/2196-1042-14-42

Nishimura, M., Chiba, M., Ohashi, T., Sato, M., Shimizu, Y., Igarashi, K., & Mitani, H. (2008). Periodontal tissue activation by vibration: intermittent stimulation by resonance vibration accelerates experimental tooth movement in rats. *Am J Orthod Dentofacial Orthop, 133*(4), 572-583. doi: 10.1016/j.ajodo.2006.01.046

Norris, R. A., Damon, B., Mironov, V., Kasyanov, V., Ramamurthi, A., Moreno-Rodriguez, R., . . . Markwald, R. R. (2007). Periostin regulates collagen fibrillogenesis and the biomechanical properties of connective tissues. *J Cell Biochem, 101*(3), 695-711. doi: 10.1002/jcb.21224

Omar, H., Shen, G., Jones, A. S., Zoellner, H., Petocz, P., & Darendeliler, M. A. (2008). Effect of low magnitude and high frequency mechanical stimuli on defects healing in cranial bones. *J Oral Maxillofac Surg, 66*(6), 1104-1111. doi: 10.1016/j.joms.2008.01.048

Ouyang, G., Liu, M., Ruan, K., Song, G., Mao, Y., & Bao, S. (2009). Upregulated expression of periostin by hypoxia in non-small-cell lung cancer cells promotes cell survival via the Akt/PKB pathway. *Cancer Lett, 281*(2), 213-219. doi: 10.1016/j.canlet.2009.02.030

Ozcivici, E., Luu, Y. K., Rubin, C. T., & Judex, S. (2010). Low-level vibrations retain bone marrow's osteogenic potential and augment recovery of trabecular bone during reambulation. *PLoS One, 5*(6), e11178. doi: 10.1371/journal.pone.0011178

Parrish, L. D., Roberts, W. E., Maupome, G., Stewart, K. T., Bandy, R. W., & Kula, K. S. (2011). The relationship between the ABO discrepancy index and treatment duration in a graduate orthodontic clinic. *Angle Orthod, 81*(2), 192-197. doi: 10.2319/062210-341.1

Pearson, O. M., & Lieberman, D. E. (2004). The aging of Wolff's "law": ontogeny and responses to mechanical loading in cortical bone. *Am J Phys Anthropol, Suppl 39*, 63-99. doi: 10.1002/ajpa.20155

Proffit, W. R. (2013). Malocclusion and Dentofacial Deformity in Contemporary Society. In W. R. Proffit, H. W. Fields & D. M. Saver (Eds.), *Contemporary Orthodontics* (5th ed., pp. 1-14). St. Louis: Elsevier Mosby.

Proffit, W. R., Fields, H. W., Jr., & Moray, L. J. (1998). Prevalence of malocclusion and orthodontic treatment need in the United States: estimates from the NHANES III survey. *Int J Adult Orthodon Orthognath Surg, 13*(2), 97-106.

Raab-Cullen, D. M., Akhter, M. P., Kimmel, D. B., & Recker, R. R. (1994). Bone response to alternate-day mechanical loading of the rat tibia. *J Bone Miner Res, 9*(2), 203-211. doi: 10.1002/jbmr.5650090209

Rakhshan, V., Nateghian, N., & Ordoubazari, M. (2012). Risk factors associated with external apical root resorption of the maxillary incisors: a 15-year retrospective study. *Aust Orthod J, 28*(1), 51-56.

Reyes, M. L., Hernandez, M., Holmgren, L. J., Sanhueza, E., & Escobar, R. G. (2011). High-frequency, low-intensity vibrations increase bone mass and muscle strength in upper limbs, improving autonomy in disabled children. *J Bone Miner Res, 26*(8), 1759-1766. doi: 10.1002/jbmr.402

Rios, H., Koushik, S. V., Wang, H., Wang, J., Zhou, H. M., Lindsley, A., . . . Conway, S. J. (2005). periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. *Mol Cell Biol, 25*(24), 11131-11144. doi: 10.1128/MCB.25.24.11131-11144.2005

Ritman, E. L. (2011). Current status of developments and applications of micro-CT. *Annu Rev Biomed Eng, 13*, 531-552. doi: 10.1146/annurev-bioeng-071910-124717

Robinson, J. A., Chatterjee-Kishore, M., Yaworsky, P. J., Cullen, D. M., Zhao, W., Li, C., . . . Bex, F. J. (2006). Wnt/beta-catenin signaling is a normal physiological response to mechanical loading in bone. *J Biol Chem, 281*(42), 31720-31728. doi: 10.1074/jbc.M602308200

Robling, A. G., Burr, D. B., & Turner, C. H. (2001). Recovery periods restore mechanosensitivity to dynamically loaded bone. *J Exp Biol, 204*(Pt 19), 3389-3399.

Robling, A. G., Hinant, F. M., Burr, D. B., & Turner, C. H. (2002a). Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. *J Bone Miner Res, 17*(8), 1545-1554. doi: 10.1359/jbmr.2002.17.8.1545

Robling, A. G., Hinant, F. M., Burr, D. B., & Turner, C. H. (2002b). Shorter, more frequent mechanical loading sessions enhance bone mass. *Med Sci Sports Exerc, 34*(2), 196-202.

Romanos, G. E., Asnani, K. P., Hingorani, D., & Deshmukh, V. L. (2014). PERIOSTIN: role in formation and maintenance of dental tissues. *J Cell Physiol, 229*(1), 1-5. doi: 10.1002/jcp.24407

Romanyk, D. L., Lagravere, M. O., Toogood, R. W., Major, P. W., & Carey, J. P. (2010). Review of maxillary expansion appliance activation methods: engineering and clinical perspectives. *J Dent Biomech, 2010*. doi: 10.4061/2010/496906

Sawada, M., & Shimizu, N. (1996). Stimulation of bone formation in the expanding mid-palatal suture by transforming growth factor-beta 1 in the rat. *Eur J Orthod, 18*(2), 169-179.

Saxon, L. K., Robling, A. G., Alam, I., & Turner, C. H. (2005). Mechanosensitivity of the rat skeleton decreases after a long period of loading, but is improved with time off. *Bone, 36*(3), 454-464. doi: 10.1016/j.bone.2004.12.001

Schauseil, M., Ludwig, B., Zorkun, B., Hellak, A., & Korbmacher-Steiner, H. (2014). Density of the midpalatal suture after RME treatment - a retrospective comparative low-dose CT-study. *Head Face Med, 10*(1), 18. doi: 10.1186/1746-160X-10-18

Sheets, C. G., Hui, D. D., Bajaj, V., & Earthman, J. C. (2013). Quantitative percussion diagnostics and bone density analysis of the implant-bone interface in a pre- and postmortem human subject. *Int J Oral Maxillofac Implants, 28*(6), 1581-1588. doi: 10.11607/jomi.3037

Srivastava, K., Tikku, T., Khanna, R., & Sachan, K. (2013). Risk factors and management of white spot lesions in orthodontics. *J Orthod Sci, 2*(2), 43-49. doi: 10.4103/2278-0203.115081

Sun, L. W., Luan, H. Q., Huang, Y. F., Wang, Y., & Fan, Y. B. (2014). Effects of local vibration on bone loss in -tail-suspended rats. *Int J Sports Med, 35*(7), 615-624. doi: 10.1055/s-0033-1358468

Taddei, S. R., Queiroz-Junior, C. M., Moura, A. P., Andrade, I., Jr., Garlet, G. P., Proudfoot, A. E., . . . da Silva, T. A. (2013). The effect of CCL3 and CCR1 in bone remodeling induced by mechanical loading during orthodontic tooth movement in mice. *Bone, 52*(1), 259-267. doi: 10.1016/j.bone.2012.09.036

Takeshita, S., Kikuno, R., Tezuka, K., & Amann, E. (1993). Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. *Biochem J, 294 (Pt 1)*, 271-278.

Tanabe, H., Takayama, I., Nishiyama, T., Shimazaki, M., Kii, I., Li, M., . . . Kudo, A. (2010). Periostin associates with Notch1 precursor to maintain Notch1 expression under a stress condition in mouse cells. *PLoS One, 5*(8), e12234. doi: 10.1371/journal.pone.0012234

Tieu, L. D., Saltaji, H., Normando, D., & Flores-Mir, C. (2014). Radiologically determined orthodontically induced external apical root resorption in incisors after non-surgical orthodontic treatment of class II division 1 malocclusion: a systematic review. *Prog Orthod, 15*, 48. doi: 10.1186/s40510-014-0048-7

Turner, C. H. (1998). Three rules for bone adaptation to mechanical stimuli. *Bone, 23*(5), 399-407.

Turner, C. H. (2006). Bone strength: current concepts. *Ann N Y Acad Sci, 1068*, 429-446. doi: 10.1196/annals.1346.039

Turner, C. H., Forwood, M. R., Rho, J. Y., & Yoshikawa, T. (1994). Mechanical loading thresholds for lamellar and woven bone formation. *J Bone Miner Res, 9*(1), 87-97. doi: 10.1002/jbmr.5650090113

Turner, C. H., & Robling, A. G. (2005). Mechanisms by which exercise improves bone strength. *J Bone Miner Metab, 23 Suppl*, 16-22.

Turner, C. H., Warden, S. J., Bellido, T., Plotkin, L. I., Kumar, N., Jasiuk, I., . . . Robling, A. G. (2009). Mechanobiology of the skeleton. *Sci Signal, 2*(68), pt3. doi: 10.1126/scisignal.268pt3

Uysal, T., Amasyali, M., Enhos, S., Sonmez, M. F., & Sagdic, D. (2009). Effect of ED-71, a New Active Vitamin D Analog, on Bone Formation in an Orthopedically Expanded Suture in Rats. A Histomorphometric Study. *Eur J Dent, 3*(3), 165-172.

Uysal, T., Gorgulu, S., Yagci, A., Karslioglu, Y., Gunhan, O., & Sagdic, D. (2011). Effect of resveratrol on bone formation in the expanded inter-premaxillary suture: early bone changes. *Orthod Craniofac Res, 14*(2), 80-87. doi: 10.1111/j.1601-6343.2011.01511.x

Uysal, T., Ustdal, A., Sonmez, M. F., & Ozturk, F. (2009). Stimulation of bone formation by dietary boron in an orthopedically expanded suture in rabbits. *Angle Orthod, 79*(5), 984-990. doi: 10.2319/112708-604.1

Vargo, J., Buschang, P. H., Boley, J. C., English, J. D., Behrents, R. G., & Owen, A. H., 3rd. (2007). Treatment effects and short-term relapse of maxillomandibular expansion during the early to mid mixed dentition. *Am J Orthod Dentofacial Orthop, 131*(4), 456-463. doi: 10.1016/j.ajodo.2005.06.032

Walker, D. H., & Wright, N. M. (2002). Bone morphogenetic proteins and spinal fusion. *Neurosurg Focus, 13*(6), e3.

Wang, S., Liu, Y., Tang, Y., Zhao, W., Li, J., Yang, Y., . . . Yu, H. (2014). Direct Radial LMHF Microvibration Induced Bone Formation and Promoted Implant Osseointegration. *Clin Implant Dent Relat Res*. doi: 10.1111/cid.12220

Watanabe, T., Yasue, A., Fujihara, S., & Tanaka, E. (2012). PERIOSTIN regulates MMP-2 expression via the alphavbeta3 integrin/ERK pathway in human periodontal ligament cells. *Arch Oral Biol, 57*(1), 52-59. doi: 10.1016/j.archoralbio.2011.07.010

Weltman, B., Vig, K. W., Fields, H. W., Shanker, S., & Kaizar, E. E. (2010). Root resorption associated with orthodontic tooth movement: a systematic review. *Am J Orthod Dentofacial Orthop, 137*(4), 462-476; discussion 412A. doi: 10.1016/j.ajodo.2009.06.021

Wysocki, A., Butler, M., Shamliyan, T., & Kane, R. L. (2011). Whole-body vibration therapy for osteoporosis: state of the science. *Ann Intern Med, 155*(10), 680-686, W206-613. doi: 10.7326/0003-4819-155-10-201111150-00006

Yee, J. A., Turk, T., Elekdag-Turk, S., Cheng, L. L., & Darendeliler, M. A. (2009). Rate of tooth movement under heavy and light continuous orthodontic forces. *Am J Orthod Dentofacial Orthop, 136*(2), 150 e151-159; discussion 150-151. doi: 10.1016/j.ajodo.2009.03.026

Yoshiba, N., Yoshiba, K., Hosoya, A., Saito, M., Yokoi, T., Okiji, T., . . . Ozawa, H. (2007). Association of TIMP-2 with extracellular matrix exposed to mechanical stress and its co-distribution with periostin during mouse mandible development. *Cell Tissue Res, 330*(1), 133-145. doi: 10.1007/s00441-007-0439-x

Zainal Ariffin, S. H., Yamamoto, Z., Zainol Abidin, I. Z., Megat Abdul Wahab, R., & Zainal Ariffin, Z. (2011). Cellular and molecular changes in orthodontic tooth movement. *ScientificWorldJournal, 11*, 1788-1803. doi: 10.1100/2011/761768

Zhang, X., Torcasio, A., Vandamme, K., Ogawa, T., van Lenthe, G. H., Naert, I., & Duyck, J. (2012). Enhancement of implant osseointegration by high-frequency low-magnitude loading. *PLoS One, 7*(7), e40488. doi: 10.1371/journal.pone.0040488

Zhang, X., Vandamme, K., Torcasio, A., Ogawa, T., van Lenthe, G. H., Naert, I., & Duyck, J. (2012). In vivo assessment of the effect of controlled high- and low-frequency mechanical loading on peri-implant bone healing. *J R Soc Interface, 9*(72), 1697-1704. doi: 10.1098/rsif.2011.0820

Zhou, Y., Guan, X., Liu, T., Wang, X., Yu, M., Yang, G., & Wang, H. (2014). Whole body vibration improves osseointegration by up-regulating osteoblastic activity but down-regulating osteoblast-mediated osteoclastogenesis via ERK1/2 pathway. *Bone*. doi: 10.1016/j.bone.2014.09.026

CURRICULUM VITAE

Mohammad Abdullah M Aldosari

Education

2014 - Master of Science in Dentistry (MSD) – Orthodontic Cert.

2009 - 2015 Doctor of Philosophy (Ph.D.), Indiana University, Indianapolis, Indiana

2000 - 2006 Bachelors of Dental Surgery (BDS) King Saud University College of Dentistry – Riyadh, Saudi Arabia

Appointments

July 2007- Clinical Instructor (on leave), Division of Orthodontics College of Dentistry, King Saud University – Riyadh, Saudi Arabia

Publications and Abstracts

Aldosari, MA., Scaramucci, T., Liu, SS., Warrick-Polackoff, JM., Zero, DT., Eckert, GJ., Hara, AT. Susceptibility of Partially-desalivated Rats to Dental Erosion by Calcium-supplemented Beverages. (International Association of Dental Research (IADR) meeting. Cape Town, South Africa, 2014)

Aldosari, MA., Chedalla, S., Fuchs, R., Liu, SS. Periostin/ OSF-2 Expression during Maxillary Suture Expansion in Mice. Abstract submitted for presentation at annual meeting of the American Association for Dental Research (AADR) meeting. Charlotte, NC(March, 2014)

Adel Al-Hadlaq, Hayder Hashim, Mohammed Al-Dosari, Ali Al-Hamad. Interrelationship Between Dental Development, Skeletal Maturity and Chronological Age in Saudi Male Children. Egypt Dent J. Vol.54, 55:65, Jan 2008