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ABSTRACT

Objective: The aim of this study is to construct a mouse orthodontic relapse model to study the effect of mechanical vibration on retention following orthodontic tooth movement (OTM). Specifically, we will apply various frequencies of vibration to the maxillary right first molar undergoing relapse to measure its effects on dental movement during the relapse phase, and monitor potential changes to the alveolar bone volume and density. We will also test for the effects of vibration on root resorption via measuring the mesial root volume.

Materials and Methods: Thirty-four male CD1 mice were randomly placed into 1 of 4 groups. The Control Group (8 mice) consisted of only OTM for 7 days. The other three groups were the experimental subset receiving OTM for 7 days, followed by 7 days of relapse. OTM was performed with mesial force application from a 10g Ni-Ti closed-coil spring connecting the maxillary right first molar and the maxillary central incisors kept in place with steel ligatures at either end, and additional composite at the incisors. Of the experimental groups, the Relapse Group (10 mice) did not receive any vibration after OTM, while Relapse + 10 Hz and Relapse + 30 Hz Groups (8 mice each) received 15 minutes of vibration from a Bose Transducer at the occlusal surface of the maxillary right first molar on days 6, 8, 10, and 13 with 10 Hz or 30 Hz vibration respectively. All animals were then sacrificed at day 14 and underwent micro-CT imaging followed by statistical analysis of bone volume fraction (BVF), tissue density (TD), first molar movement (M1-M2 Distance), and mesial root volume (MRV).

Results: Differences in M1-M2 Distance were statistically significant between control and experimental groups. No significant findings were observed between controls and experimental

groups for BVF, TD, and MRV. No significant differences were observed between relapse and relapse with vibration groups for BVD, TD, M1-M2 Distance, and MRV. The M1-M2 Distance data showed that Relapse + 10 Hz vibration demonstrated 50% decrease in retention, while maximum retention was observed in Relapse + 30 Hz vibration, demonstrating 50% increase in retention compared to Relapse Group only. Trends in increasing BVF and TD were also observed for Relapse + 30 Hz vibration compared to Relapse only group.

Conclusion: Our findings demonstrate that 10 Hz and 30 Hz mechanical vibration have no statistically significant effects on retention based on a mouse relapse model. However, trends in the data demonstrate positive potential for 30 Hz vibration in anabolic bone formation and decreasing relapse rate. Further research with increased samples sizes, dosages, modes, and frequencies of vibration is necessary to shed light upon the effects of mechanical vibration on retention.

BACKGROUND

Introduction

Retention is a phase of orthodontic treatment that serves to maintain teeth in their final positions after active tooth movement [1]. Usually, teeth have a tendency to relapse back to their original positions following active movement [2]. Some of the different types of relapse observed following fixed orthodontic therapy include decreases in arch length and intercanine width, and increase in mandibular crowding [3, 4]. Interestingly, Little et al. performed analysis of longitudinal changes in anterior alignment of the mandibular dentition for 10-20 years, demonstrating that the maximum relapse occurred during the first 10 years post-retention, and continued into 20 years, with only 10% of treatments remaining clinically satisfactory [3]. Relapse has been thought to be multifactorial in nature, often involving the origin of malocclusion, bone turnover, periodontal factors, soft tissue forces, growth, and function [5, 6]. Due to the diverse elements involved, relapse remains incompletely understood, giving rise to wide variations in retention protocols amongst clinicians [6].

Retention has been a controversial topic since the early stages of the specialty; Angle in 1907 stated that "the problem involved in retention is so great…greater than the difficulties being encountered in the treatment [7]." Calvin Case later added in 1920: "the very cases which create in us the greatest pride, are going back to their former malpositions and disharmonies, in spite of everything we have been able to do with retaining appliances [8]." Later, McCauley debated that the transverse width of the canines and molars determine stability in 1944, while Tweed reported the inclination of the incisors was the important factor in retention [9, 10]. In a landmark article in 1988, Little et al., mentioned above, declared that "the only way to ensure continued satisfactory alignment post-treatment probably is by use of fixed or removable retention for life." Later in 1999, Little further retrospectively reviewed a collection of over 800 patients from

University of Washington for relapse, and concluded that (1) Arch length decreases after orthodontic treatment. (2) Arch width measured across the mandibular canine teeth typically reduces post-treatment, whether or not the case was expanded during treatment. (3) Mandibular anterior crowding during the post-treatment phase is a continuing phenomenon well into the 20-to-40 years age bracket and likely beyond. (4) Third molar absence or presence, impacted or fully erupted, seems to have little effect on the occurrence or degree of relapse. (5) The degree of post-retention anterior crowding is both unpredictable and variable and no pretreatment variables either from clinical findings, casts, or cephalometric radiographs seem to be useful predictors either before or after treatment [11]. Finally, Littlewood et al. concluded in their systematic review in 2006 that "There is currently insufficient evidence on which to base the clinical practice of orthodontic retention." [5]

Nature of Relapse

There are multiple theories regarding the causes of relapse. Proffit mentions according to the Equilibrium theory that muscle and soft tissue pressures on the occlusion may be driving causes of relapse [12]. Another school of thought concentrates on the periodontal ligament (PDL), suggesting that forces exerted by stretched periodontal and gingival connective tissue fibers may place tensile forces on moved teeth or that collagen fibers of the PDL are leading causes of relapse [13, 14]. The role of the supracrestal fibers of the PDL was later questioned and Edwards demonstrated that circumferential supracrestal fibrotomy (CSF) may also aid in retention [15]. On a separate note, Tweed explained that changes in tooth inclination, such as proclination of the incisors, are important for stability in 1944 [10]. Gianelly later argued that intercanine distance, especially in the mandible is important in preventing relapse [16]. The amount of continuous

bone turnover has also been associated with relapse, as well as continuous growth, especially in the vertical dimension [17, 18]. Other theories include continuous, interproximal force, originating in the periodontium and acting on adjacent teeth at their contact points, as proposed by Southard et al. [19]. In a review of literature performed in 1998, six major criteria for the stability of finished orthodontic cases were identified: 1) pretreatment lower arch form should be maintained, 2) Original lower intercanine width should be maintained; expansion of this is the most predictable of all relapses, 3) mandibular arch length decreases with time, 4) the most stable position of the lower incisors is their pretreatment position, 5) Fiberotomy is an effective means of reducing rotational relapse, and 6) Lower incisor reproximation can improve long-term post-treatment stability [20].

The application of orthodontic force for tooth movement causes compression sides and tension sides of the affected teeth. This strain in turn induces an inflammatory response in the surrounding tissues, producing a cellular response of macrophages, osteoclasts, osteoblasts, and fibroblasts to the periodontium and bone to induce remodeling [21, 22]. The compression side of tooth movement involves osteoclastic activity resulting in bone resorption while osteoblastic activity occurs at the opposite tension side where new woven bone is deposited [21]. After removal of fixed orthodontic treatment, the new woven bone is remodeled and replaced with mature lamellar bone. Lamellar bone has characteristics of being more organized and having higher mineral content and strength than woven bone thus making it less susceptible to resorption [23]. Remodeling may initiate relapse of teeth due to a temporary void and absence of lamellar bone adjacent to the PDL. This in conjunction with rebound forces of the PDL can promote relapse [24, 25]. Furthermore, other tissues remodel around the teeth; the PDL reorganizes over 3-4 months, gingival collagen fiber networks remodel in 4-6 months, and

supracrestal fibers exceed even 7 months of remodeling [26]. A retention period of over 12 months allows ample time for remodeling of each type of tissue described above [27].

Review of Retention Studies

Orthodontists utilize various appliances to maintain dentition in their final positions following OTM. The most common appliances are Hawley retainers, Vacuum-formed retainers, and permanent fixed retainer. Pratt et al. performed a survey of the American Association of Orthodontics members in 2011 which revealed that in the maxillary arch, the Hawley retainer is used most by 47% of their sample, followed by the vaccum-formed retainer 41%, and permanent fixed retainer at 11%. In the mandibular arch, these devices were utilized 29%, 29% and 42% respectively [28]. The Hawley retainer consists of an acrylic base and labial bow of stainless steel wire which can be adjusted according to provider preference [29]. Its advantage is that it allows settling of the posterior teeth, if no occlusal impediments are provided such as an Adam's clasp and teeth are allowed to erupt freely. A disadvantage of the Hawley retainer is its extensive palatal coverage [30]. The vacuum-formed retainer, commonly known as the Essix retainer, is a removable retainer that is constructed out of various thicknesses of plastic and covers all surfaces of the teeth. Its advantage is that it is nearly invisible and has no palatal coverage. Its disadvantage is that it does not allow for occlusal settling and that it is less durable [30]. Despite the mechanical design of these removable appliances, the limitation to their function is patient compliance, although the Hawley has been reported to be more successful in compliance with patients over two years in retention [30, 31]. Due to majority of orthodontic patients falling within the adolescent and teenage years, variable rates of compliance are achieved and can be very discouraging for clinicians [32]. Also, patient compliance with retainers decreases over time, with fewer than half of the patients wearing them as instructed

following completion of active treatment [33]. Interestingly, more than 50% of patients admitted that they did not wear retainers as instructed, with the most common reasons being discomfort and forgetfulness [34].

Fixed retainers have been utilized to manage compliance problems in patients. Fixed retainers are often placed lingual to the mandibular anterior dentition, and are often bonded from canine to canine to prevent relapse of the mandibular incisors [30, 35]. Although compliance problems are eliminated, the technique has some disadvantages [28]. The wire has to be passive, and oral hygiene is a concern [30]. Pandis et al. have shown higher calculus accumulation, greater marginal recession and increased probing depths in a group of patients with mandibular lingual fixed retention. This study emphasized careful selection of retention protocols after a thorough consideration of anatomic, hygiene, social, and cultural factors followed by close monitoring of patients [36].

Less popular retention strategies include surgical-based interventions such as frenectomies or circumferential supracrestal fibrotomy (CSF). For example, patients with a large maxillary anterior diastema and labial frenum attachment to the alveolar ridge penetrating from buccal to lingual benefit from a frenectomy to relieve the fibers inhibiting stability of diastema closure [27]. Often frenectomies are recommended to be performed after closure of the diastema to prevent scar tissue from hindering closure [23]. CSF targets the supracrestal fibers that contribute to the tensile forces that cause relapse and take a considerable amount of time to remodel [27]. This procedure consists of the surgical transsection of supracrestal free gingival fibers surrounding the tooth, and has been shown to decrease the relapse of teeth [13]. Fiberotomy has been shown to be effective in retention, especially in preventing pure rotational relapse rather

than reduction of labiolingual relapse over the long term, and its results are better in the maxillary anterior region than in the mandibular anterior region [15].

Other views regarding relapse include theories that compressive interproximal force, originating continuously from the periodontium and acting on adjacent teeth at their contact points may be responsible for some long term arch constriction and decreased stability [19]. Furthermore, Southard et al. found significant correlation between mandibular anterior alignment and interproximal force. This is potentially due to the narrower contacts of the lower incisors, and broadening them can resist contact slippage and increase stability [30]. Another suggested mode of treatment to relieve this force was prophylactic extraction of third molars, which has currently been rejected as a mode of treatment [37, 38]. In addition, based on the type of relapse, overcorrection may help in retention. Anterior-posterior overcorrection of the occlusal relationship is recommended in Class II patients, as is overextrusion of anterior teeth in open bite cases, and overintrusion in deep bite cases [23, 39].

Kim et al. investigated the effectiveness of pharmacological agents in preventing orthodontic relapse in rats, showing that systemic administration of the bisphosphonate, pamidronate significantly inhibits initial relapse of mesialized molars by inhibiting osteoclastic activity. They attempted to create a relapse model by placing elastic bands between the first and second molar of rats, and applied injections of bisphosphonates at the site after band removal to observe this trend [40]. Hassan et al. created another relapse model in sheep in 2010 where they extracted central incisors and tipped lateral incisors and used Bone Morphogenetic Proteins (BMPs) to evaluate the post-orthodontic stability. They discovered less relapse when injecting BMPs into the PDL of tipped incisors as compared to the controls. Active bone remodeling and hypercementosis were also observed [41]. Statins have also been shown to stimulate osteoblastic

activity which would theoretically strengthen the bony housing of teeth and prevent their relapse. In 2010, Han et al., explored the effects of simvastatin on a rat relapse model, where springs were placed at the molars and incisors bilaterally to induce movement of the molar, and the drug was injected systemically. They observed increased retention potential and increased osteoprotegerin (OPG) levels [42]. OPG is an osteoclast inhibitor that binds to receptor activator of nuclear factor κβ Ligand (RANKL) and functions as a competitive inhibitor of the RANK receptor necessary for osteoclast activation, thus inhibiting osteoclastogenesis and bone resportion [25]. Hudson et al. utilized the same rat relapse model as Han et al., but administered OPG adjacent to the molars, observing over close to 50% decrease in relapse in low dosage OPG groups and over 50% decrease in relapse in high dosage OPG groups compared to controls [25]. Limitations to pharmacological agents include local delivery and prevention of systemic side-effects, as well as pain and discomfort. Zhao et al. addressed controlling local delivery of drugs in 2010, utilizing a rat model with a spring force between the right maxillary first molar and incisors. They utilized local OPG gene transfer with inactivated hemagglutinating virus and OPG expression plasmid to periodontal tissues at the molar. The OPG was injected into the palatal mucosa on the distal surface of moved tooth and the percentage of relapse in the experimental groups was significantly less than in the control group (35.7±8.9% versus 96.3±7.0%). They also tested for systemic effects via monitoring inflammation at the tibia, and saw no changes. Kanzaki et al. performed a similar OPG gene transfer in a relapse model in rats, where a compressive 17 gram spring was placed between first molars palatally to tip them, and injection at the site was made to test retention. This group reported almost half the amount of relapse observed compared to controls [43]. While this method delivers promising results, gene transfer may cause severe immunologic reactions to the inactivated virus as well as accidental

activation of oncogenes, forming neoplasms, or accidental diffusion from palatal to buccal surfaces during injections [43, 44].

Pharmacological methods of tooth stability, though promising in theory, have several limitations. The majority of methods under research are in animals models, have systemic effects, require questionable methods of delivery, and lack of long term data. Currently, mechanical retention (i.e. retainers) is the suggested and most popular mode of retention clinically. Development of an adjunct to retainers would minimize the time needed to wear retainers and increase the overall stability of OTM. Studies relating retention with vibration are limited. Vibration would serve as a potential minimally invasive adjunct to mechanical retention to minimize orthodontic relapse.

Vibration Research

Studies of whole-body vibration have been performed in both animal and humans models. Christiansen and Silva studied the effect of this type of vibratory stimuli on 40 adult mice using a frequency of 45 Hz with varying magnitudes of force for 15 minutes per day in a 5 week interval. They found an increase in trabecular bone volume in the experimental vibration group, independent of dosage [45]. Rubin et al. performed a 1-year prospective, randomized, double-blinded, placebo-controlled clinical trial on seventy post-menopausal women. In these subjects they administered whole-body vibration at a frequency of 30 Hz with 0.2 grams of magnitude for twenty minutes per day. They found an inhibition of bone loss in both the spine and the femur with pronounced findings associated with lower body mass [46]. These studies promoted that low-magnitude, high frequency vibration for relatively short durations has an anabolic potential for bone, with findings mostly demonstrating increased numbers and sizes of trabeculae, with improved stiffness and strength of cancellous bone [46]. Since the molecular mechanisms

involving bone turnover, specifically modeling and remodeling, are similar to those required for OTM, applying vibratory stimuli might have an effect on the rate of tooth movement.

Other studies have looked at applying a pulsed electromagnetic field (PEMF) in order to create a vibratory stimulus. Stark and Sinclair looked at applying PEMF in 40 male Hartley guinea pigs, where they measured the effects of 25 Hz PEMF with 12 grams of orthodontic force for ten days. They observed an overall increase in the rate and amount of tooth movement along with greater bone matrix deposition and numbers of osteoclasts [47]. Darendeliler, Sinclair and Kusy in 1995 also studied the effects of PEMF along with a samarium-cobolt magnet, applying 15 Hz vibration with 15 grams of orthodontic force for 10 days. They concluded that the amount of OTM in the magnet and PEMF groups was greater than the group with orthodontic force alone [48]. They proposed that the change in the rate of OTM was due to a reduction of the initial lag phase which follows force application [48]. Darendeliler et al.then further investigated the effects of PEMF and neodymium-iron-boron magnets in 45 Wistar rats. They applied 25 grams of orthodontic force with a frequency of 30 Hz and demonstrated significantly greater OTM in the group exposed to PEMF vibration [49].

Other types of vibration studied also include resonance vibration (with continuously changing frequency) and ultrasonic vibration. Nishimura et al.tested the effect of resonance vibration on OTM in 42 Wistar rats divided in two groups over 21 days. A 0.012 nickel-titanium (Ni-Ti) expansion spring with 12.8 grams of orthodontic force was applied with and without weekly 8 minute resonance vibration (60 ± 8 Hz) session to the occlusal surface of 1st molars. They concluded that there was 15% greater OTM rate with combined resonance vibration and force, and histologically noted greater receptor activator of nuclear factor kappa-B ligand (RANKL) expression by osteoclasts and fibroblasts on day 3, with increased numbers of osteoclasts present

(1.7x control) on day 8 [50]. Similarly, Ohmae et al. researched ultrasonic vibration in a split-mouth model on 5 adult male beagle dogs with bilaterally extracted maxillary first premolars. An eighty gram force using a sectional archwire between the canine and first premolar was applied to close the extraction space, with one side exposed to homo-directional ultrasonic vibration (2 minute interval, two times per week for a total of 8 – 10 weeks) while the other side served as a control. They also identified a greater amount of tooth movement in the teeth exposed to vibration [51].

Throughout the last few years, the AcceleDent^{TM} company has produced a device that can be used in humans in order to apply a vibratory force of 30 Hz to the dentition with two corresponding studies. The first study was a non-controlled experiment in 14 subjects for 20 minutes of appliance use per day over a total of 6 months. While no controls were used, they postulated that the observed 3mm per month of tooth movement in the maxilla and the 2.1mm per month in the mandible were greater results compared to current clinically accepted norms (approximately 1mm tooth movement per month) [52]. Following these findings, they conducted a prospective, randomized, blinded, sham-controlled clinical trial on 45 human subjects at the University of Texas at San Antonio, with promising results pending publication. They found significantly greater tooth movement during the aligning phase (106%) and significantly greater tooth movement during space closure (38%). On the contrary, data regarding the effect of mechanical vibration on OTM in an animal model at the University of Connecticut Health Center have demonstrated different results [53]. Twenty six female Sprague Dawley rats were divided into four groups: Control un-loaded, Vibration, OTM of 25 grams mesial load, and OTM with vibration with 0.4 N and 30 Hz twice per week for 10 minutes. Rather than increases in OTM, cyclical forces inhibited the amount of OTM with histological

analysis showing disorganization of collagen fibril structure of the PDL, and increased osteoclast parameters with significant decrease in bone volume fraction in the molar region.

OTM Models and Retention Model

Several animal models have been designed to study tissue responses to mechanical loading during orthodontic tooth movement. Primate, dog and cat models have been reported in initial histological studies using light microscopy [54, 55] and electron microscopy [54, 56]. The rat model proposed by Waldo in 1954 [57] had increased levels of experimental control over other animal models and has become the investigative approach for researching the processes of mechanotransduction and alveolar bone remodeling in OTM [58]. Currently, rats are most commonly used, accounting for over half of all orthodontic tooth movement animal studies [58]. Compared with most other animals, the use of the rat model has several advantages: it is relatively inexpensive, which allows using large sample sizes, longer housing periods allow for longer duration of experiments, histological preparation of the rat is easier than other animal models, there is greater availability of antibodies required for cellular and molecular biological techniques, and their sizes are larger than mice, allowing for easier placement of orthodontic appliances. Yet, the rat model has some limitations: denser alveolar bone as compared to humans, lack of osteons and less abundant osteoid tissue, structural dissimilarities in the arrangement of PDL fibers and the supporting structures, and faster tissue development during root formation and changes incident to orthodontic treatment than in humans, while maintaining relatively the same principle mechanisms [58].

Rat models provide for a diverse scope of orthodontic research ranging from measuring proliferation rates of PDL cells under load to assessing the effects of prostaglandins,

bisphosphonates and leukotrienes on tooth movement [59-61]. Ren et al.'s systematic review of the 153 (57% of the total tooth movement models) studies performed on rats in the past twenty years determined that the majority of the experimental models designed poor force systems that lacked controls throughout the duration of tooth movement [58]. Only three methods met Ren's inclusion criteria for a good model [58]: a force magnitude of less than 20cN; mesial movement of molars; an experimental duration greater than 2 weeks without other experimental conditions, such as drug intervention. Most of the studies did not consider physiology of the rat (i.e. natural distal drift of the molars and continual eruption of the incisors), nor faulty appliance design. The distal drift of the molars underestimates the amount of mesial movement of the molars with continual eruption of the incisors leading to minimized control of force direction. The appliance design is poor when the 50 fold decrease in rat molar root surface area is not considered compared to humans, or if there is a lack of constant and continual force [58].

Pavlin et al. (2000) first developed a mouse model for testing the load conditions necessary to generate an optimal biological response of paradental tissues [62, 63]. They used an elastomeric "o-ring" tied between maxillary incisors and the first molar, and a red elgiloy (alloy of nickel and cobalt) open coil spring (0.0056 x 0.022 inches, Rocky Mountain Orthodontics, Denver, CO) tied and bonded to the same teeth, respectively. It was found that the coil spring has considerable advantages over the "o-ring." Firstly, bonding of a coil spring to the molar and the incisors eliminates contact of the appliance with gingival tissues, greatly reducing the risk of tissue irritation [62, 63]. This correlated with the criticisms of Charles Waldo, whom in 1954, was among the first pioneers responsible for the advent of the rat model. The Waldo method utilized an orthodontic intermaxillary elastic, which was stretched and inserted into the interproximal space just cervical to the contact between the molars of rats [57]. This method has been

criticized due to the unknown force decay of the elastic. Springs have proven to be more reliable due to delivery of a reproducible force of 10±2 cN over a range of 3-15 mm of activation [58]. Secondly, the spring has a lower force/deflection rate (F/Δ). This allows for a more precise and reproducible application of a low level force, which also remains more constant compared with that delivered by an elastomeric "o-ring."

In 1990's, King [64], Keeling [65], and Nixon [66] met al.l of Ren's criteria for an ideal rat model [58]. Forces of 20, 40, and 60 cN were used in all 3 articles. These studies were criticized for having an initial constant force without proper reactivation, as well as forces of 40 and 60 cN as too heavy. The appliance consisted of a 9 mm length of closed coil spring (0.006 inch NiTi; arbor diameter: 0.022 inch, Unitek, Monrovia, Calif.) suspended between a cleat bonded to the occlusal surface of the maxillary first molars and the lateral surface of the maxillary incisors. Initial force values were measured by suspending known weights from the anterior end of these coils prior to fixation to the incisors. Tooth movement was based on enlarged cephalograms, and was measured from the position of a reproducible landmark on the molar cleat with respect to either zygomatic amalgam implants, or a barbed broach placed submucosally on the palate. Palatally placed barbed broaches represented a more reliable, less traumatic, and more easily executed superpositional landmark than zygomatic amalgams. They only had a 79% appliance success rate, the animals lost weight, and they extracted mandibular first and second molars. All of these factors contributed to poor overall animal care [58, 64-66].

Finally in 2004, Ren's model was fabricated due to the shortcomings of previous rat models, and used a spilt-mouth design. This design compensated for the physiological distal drift of the molars, growth of the snout and forward movement of the incisors, and the continuous eruption with possible distal tipping of the incisors. Stainless steel ligature wires with a diameter of 0.2

mm were bent to enclose all three maxillary molars as one unit. To this ligature wire a Sentalloy® closed coil spring (Ni Ti, 10 cN, wire diameter 0.22 mm, eyelet diameter 0.56 mm, GAC, New York, USA) was attached to deliver a reproducible force of 10 ± 2 cN over a range of 3-15 mm activation. A transverse hole was drilled through the alveolar bone and both maxillary incisors at the mid-root level using a drilling bur (D0205, Dentsply). A stainless steel ligature wire (diameter 0.3 mm, Dentaurum) was inserted through the hole and bonding was applied until the buccal and palatal wires were fully embedded in the bonding material prior to light curing. The coil was activated and attached to the ligature wire through the snout and the incisors [58].

Recently in 2006, Yoshimatsu et al. used a variation of the Ren model with Ni-Ti closed coil springs [67] in order to further develop the mouse model for OTM. Their mouse model included a Ni-Ti closed coil spring with wire diameter of 0.15mm and coil diameter 0.9mm. The appliance was inserted between the maxillary incisor and the first molar on the left side. It was fixed with a 0.1mm wire around each tooth using a dental adhesive agent (Superbond; Sunmedical Shiga, Japan). To prevent detachment of the maxillary incisors during the experiment, a shallow groove, 0.5mm from the gingiva, was made on the maxillary incisor every 4 days, and the wire was reattached at the new groove with 10 grams of force after activation. The maxillary left molar was used as the experimental side, and the right as the control, taking into account the distal molar drift that would naturally occur [67]. Our experimental models will utilize the above mentioned advances in OTM in mice to construct a reproducible model for OTM.

We decided to perform our experiments in mice due to several reasons. Mice are commonly used for studies of skeletal biology due to their similarity to humans when investigating genetic or molecular factors, and the National Human Genome Research Institute has confirmed that

overall, mice and humans share almost every gene in a closely related form. Of the approximately 4,000 genes that have been studied, less than 10 are found in one species but not in the other (5). Also, murine strains allow for proteomics studies, which help elucidate functions of different cells, signaling pathways, secondary mediators, and transcription factors at a molecular level, for example, in retention and OTM (94). Furthermore, mice reproduce quickly, and are cheaper to house, grow, and maintain. Finally, utilizing a certain strain of cloned mice (CD-1 for example), we have a genetically and phenotypically homogenous sample.

As for an orthodontic retention model, several models exist that were mentioned in the retention studies section above [14, 25, 40-44]. The Waldo method has been utilized where a rubber band elastic is placed between the first and second molar of rats, and after a set period of time, is removed and relapse is noted [14, 40]. Sheep maxillary central incisors have also been extracted and lateral incisors were tipped mesially, followed by a period monitoring their relapse [41]. Rat molars have also been tipped palatally using a palatal spring appliance, and then studied for relapse [43]. Mesial movement of rat molars with a spring has been studied unilaterally and bilaterally as well [25, 42, 44]. For example, Hudson et al. utilized a Sprague-Dawley rats relapse model divided into two phases: initial tooth movement (days 1–28), during which springs were placed between the incisors and maxillary first molars bilaterally, and the tooth relapse phase (days 28–52), during which injections of OPG or PBS were administered throughout the molar relapsing phase. Zhao et al. performed a gene transfer study where they separated the right palatal rat first molar mesially and unilaterally with a spring, and performed OPG gene transfer to measure relapse results [44]. Our model is a modified version of the Zhao et al. model, where we attach our spring at the incisors and the right maxillary first molar of mice unilaterally, and provide vibration at different frequencies with the same dosage interval during the relapse phase

in a shorter experimental timespan. Our experimental model is the first relapse model utilizing mice for retention in conjunction with vibration.

RATIONALE

One of major limitations to orthodontic treatment is relapse after active OTM. Relapse occurs often, and retention strategies require patient compliance for effectiveness. Introduction of a simple protocol for prevention of relapse would have large implications in clinical orthodontics, and help to maintain the final outcomes of orthodontic treatment. Due to the multifactorial nature of relapse, research to decrease relapse rate is difficult and scarce. Currently, orthodontic animal retention models have been primarily used for pharmacological studies on relapse [14, 25, 40-44]. While pharmacological studies present promising results on animal models, their drawbacks include systemic effects and uncertainties in drug delivery vectors that limit their application clinically on humans. Therefore, less invasive procedures should be developed to limit orthodontic relapse and the need for long-term wear of retainers. Cyclical loading, especially at a frequency of 30 Hz, has been studied in the past several decades in bone turnover, and has demonstrated anabolic bone formation with confirmed results. Recently, new vibration devices, including a popular unit that uses 30 Hz frequency vibration, have been released for use clinically in orthodontics, and advertise faster tooth movement. Nevertheless, research has demonstrated conflicting data regarding the effects of such appliances, with decreases in the rate of OTM observed in some studies. Since decreasing OTM would promote retention of teeth, and previous studies have confirmed anabolic activity for vibration, we decided to examine changes in orthodontic relapse with different dosages of vibration. We will be the first to launch a relapse study utilizing vibration in a mouse model. The objective of our controlled study is to evaluate

the effect of various frequencies of cyclical loading on the rate of retention, bone quality, and root resorption in a mouse dental relapse model following OTM.

HYPOTHESES

Hypothesis 1: We hypothesize that the application of vibration shortly prior to finishing OTM and continued after removal of force will decrease the rate of relapse (tooth movement) of the teeth.

Hypothesis 2: We hypothesize that the application of vibration shortly prior to finishing OTM and continued after removal of force will induce notable changes in bone density around the teeth to aid in increased retention.

Hyposthesis 3: We hypothesize that the application of vibration shortly prior to finishing OTM and continue after removal of force will prevent root resorption of the tooth undergoing relapse.

Null Hypothesis 1: There will be no difference in retention (movement) of teeth after OTM in our relapse model with vibration groups compared to the non-vibration relapse group.

Null Hypothesis 2: There will be no difference in the bone quality at the tooth undergoing relapse in the vibration groups compared to the non-vibration relapse group.

Null Hypothesis 3: There will be no difference in root resorption of the tooth undergoing relapse in the vibration groups compared to the non-vibration groups.

SPECIFIC AIMS

Specific Aim 1: To utilize an in vivo mouse model to measure the effects of two different frequencies of vibration on retention (movement) of a tooth undergoing relapse.

Specific Aim 2: To determine the effects of two different frequencies of vibration on bone quality at the site of relapse of a tooth relapsing after OTM.

Specific Aim 3: To determine the effects of two different frequencies of vibration on root resorption of a tooth undergoing relapse.

MATERIALS AND METHODS

Study Design

All experimental procedures were performed at the University of Connecticut Health Center under the strict guidelines of an approved protocol (ACC# 100340-0115) for animal experimentation. The study consisted of 34 male CD1 mice (12 weeks old), which were randomly placed into 1 of 4 groups (1 control/ 3 experimental). In each group, the procedure was applied to the right side of the maxilla. OTM of the maxillary first molar was performed via an orthodontic force from a spring for 7 days. Relapse groups required removal of the spring after 7 days, allowing for 7 days of additional relapse of the right maxillary first molar. Additional mechanical vibration of the maxillary first molar, if applied, was performed at the end of the OTM phase, and throughout the relapse phase of the experiment.

The following is the control group:

(1) OTM (Control group-8 mice)

The following are the 3 experimental groups:

(1) OTM + relapse (Relapse group-10 mice)

(2) OTM + relapse + 10 Hz Vibration (Relapse + 10 Hz group-8 mice)

(3) OTM + relapse + 30 Hz Vibration (Relapse + 30 Hz group-8 mice)

Method for Orthodontic Force Application

Animals were anesthetized with an intraperitoneal injection of ketamine and xylazine (6μL/g body-weight). A custom mouth-prop was fabricated from 0.032 mm SS wire and was placed between the maxillary and mandibular incisors in order to hold the mouth open.

OTM required subjecting the mice to an orthodontic force via a Nickel-Titanium (Ni-Ti) coil-spring placed between the central incisors and the maxillary right first molar. Specifically, a low force/deflection rate Ni-Ti closed coil-spring (G&H wires, Indianapolis, IN) was placed and activated 1.5mm delivering a continual force of approximately 10g (Figure 1). The force/deflection rate (F/Δ) for the spring was determined in order to calibrate the amount of force produced by activation of the spring.

Prior to appliance delivery, Ni-Ti coil spring appliances were pre-fabricated consisting of two separate segments of 0.004 inch stainless-steel (SS) 304 V annealed ligature wire (Xylem Company, Fort Wayne, IN), one connected to either end of the Ni-Ti coil spring (wrapped around two coils for stability).

In order to connect the spring appliances, one end of the spring was connected to the molar and the other end of the spring was connected to the incisors utilizing the 0.004 inch SS ligature wire. At the molar, 0.004 inch SS ligature wire was threaded through the contact between the first and

second right maxillary molars from buccal to palatal, wrapped and tightened around the first molar, and cinched below its height of contour on the palatal side. The spring was then activated to the incisors with the 0.004 inch SS ligature wire wrapped tightly around both maxillary central incisors. The maxillary incisors were notched disto-gingivally. To prevent any dislodging of the ligature and spring, the ligature wire around the incisors was secured into the disto-gingival notches using composite resin (Transbond XT Light Cure Adhesive Paste, 3M Unitek, Monrovia, CA), which was cured using a commercial LEDemetron-1 unit (Dentsply, York, PA) following a round of etching (Reliance Ortho Prod Inc, Itasca, IL), washing, drying, and application of Assure Bonding Agent (Reliance Ortho Prod Inc, Itasca, IL). Finally, the mandibular incisors were reduced 2mm in length incisally to decrease appliance breakage and failure when the mice were masticating [67].

After appliance insertion, the mice were allowed to recover in the presence of an incandescent light for warmth, and then returned to their cages once full ambulation, function, and self-cleansing had returned. The appliance was checked every day to ensure optimal force delivery for OTM, and additional bonding material was added if necessary. After completion of day 7, all intraoral appliances (ligatures and spring) were removed. The mice then continued the final 7 days of the experiment without any intraoral appliances, allowing for relapse of OTM. The duration of the experiment was 14 days.

Application of Mechanical Vibration

Following adequate induction of general anesthesia using a mixture of ketamine and xylazine (described above), a custom mouth-prop fabricated from 0.017" x 0.025" Titanium Molybdenum Alloy (TMA) wire was placed between the maxillary and mandibular incisors in order to hold

the mouth open. At this point, a feedback-loop controlled electromechanical actuator (Model 3230, Bose/EnduraTec, Minnetonka, MN) was utilized in order to apply unilateral mechanical vibration to the occlusal surface of the maxillary right first molar along the long axis of the tooth, with a loading force of 1g (Figure 2). Loading protocols for individual animals consisted of 15 minutes of mechanical vibration at 10 or 30 Hertz (cycles/second) depending on the experimental relapse group. Mechanical vibration was applied at days 6, 8, 10, and 13 (Figure 3).

Wellness Monitoring and Euthanasia

Depending on the group assignment, mice were exposed to orthodontic force, mechanical vibration, or the combination of both. Prior to any experimentation, all mice were acclimated to a 12-hour light/dark cycle for at least 1 week.

All animals were housed under normal laboratory conditions and were fed a soft powder diet (Bio-Serve Frenchtown, NJ) and water ad libitum. In order to monitor the food intake during the experiment, all mice were weighed every 3 days. Any mouse that lost more than 20% body-weight was sacrificed and excluded from the study.

Upon completion of the experiment (day 14), all mice were euthanized by CO_2 inhalation. All animal experimental procedures were in compliance with the guidelines set forth in the Guide for Care and Use of Laboratory Animals [68].

Micro-CT Analysis and Tooth Movement Measurements

Following euthanasia, at day 14, the mice were decapitated and cleansed of soft tissues. The skulls were then placed in 10% neutral buffered Formalin for seven days at +4°C with constant agitation, upon which time they were sent for radiographic imaging. Specifically, three-

dimensional images were obtained using a micro-focus X-ray computed tomography (micro-CT) machine. All micro-CT imaging and subsequent analysis was performed by the Micro-CT facility, located in The Medical Arts and Research Building (MARB) at the University of Connecticut Health Center.

Scanning was performed at 55 kV and 145 amps, collecting 1,000 projections per rotation at 300 millisecond integration times. Three-dimensional images were then constructed using standard convolution and back projection algorithms with Shepp and Logan filtering and rendered within a 12.3 mm field of view at a discrete density of 578,704 voxels/mm³ (isometric 12 mm voxels).

The images obtained were then utilized to determine the amount of orthodontic tooth movement by measuring the distance between the right maxillary first and second molars. The two points that were used were the most distal point of the first molar (M1) and the most mesial point of the second molar (M2), with the difference (M1-M2 distance) being the total distance the tooth has moved as in the control group, or the total distance left between the molars after relapse following removal of orthodontic force seen in the experimental groups. These measurements were made in the sagittal plane along the path of the tooth movement, which was located by determining which image plane showed the most root structure.

The region of interest for the analysis of bone volume fraction (BVF) and tissue density (TD) consisted of a square region that extended 200 μm from the mesial surface of the disto-lingual root to the distal surface of the mesio-buccal root of the right maxillary first molar (Figure 4). The mesial root volume (MRV) was also measured to check for resorption due to OTM.

STATISTICAL ANALYSIS

Descriptive statistics were used to examine the distribution of BVF, tissue density, first molar movement, and mesial root volume. A One-Sample Kolmogorov-Smirnov test was used to

examine the normality of data distribution. Outcomes were compared between control, relapse, relapse + 30Hz, and relapse + 10 Hz groups using One-way Analysis of Variance (ANOVA) and Kruskal Wallis test where applicable. Multiple pair-wise comparisons were conducted to examine differences in outcomes between the control group and the treatment groups, and amongst treatment groups themselves. In order to minimize the possibility of Type 1 errors due to multiple pair-wise comparisons, Bonferroni adjustments were conducted. For each outcome, a total of six pair-wise comparisons were conducted. The p-value was set at 0.008 to be statistically significant. All statistical tests were two-sided. SPSS Version 22.0 (IBM Corp, NC) software was used to conduct the data analysis.

RESULTS

All 34 mice included in the study remained healthy and had a slight increase in body weight by the end of the experiment. There was no loss of the spring or breakage of the ligature wire throughout the entire experiment.

One-Step Kolmogorov-Smirnov Test to analyze samples for normality indicated that BVF and MRV were distributed normally (parametric) while tissue density and first molar movement were not normally distributed (non-parametric) as seen in Table 1. The overall distribution of BVF (at region of interest), tissue density, M1-M2 distance (distance between the right maxillary first and second molar), and MRV by treatment groups are summarized in Table 2. Direct comparisons between control and experimental groups for BVF, TD, and M1-M2 distances, and MRV are summarized in Table 3. There were no significant differences in BVF, TD, and MRV amongst the control and treatment groups, while M1-M2 Distances demonstrated significant differences between the distribution of data across control and experimental groups (Table 3). Multiple pair-wise comparisons for the four outcome measures are summarized in Tables 4 to 7 for all data.

The pair-wise comparisons showed that there were significant differences in M1-M2 Distance between Control and Relapse group (p=<0.0001), Control and Relapse + 10 Hz group (p<0.0001), and Control and Relapse + 30 Hz group (p=0.002) even after Bonferroni corrections. No other statistically significant pair-wise comparisons were observed between the control and experimental groups. Comparisons of data for controls with experimental groups are shown in Figures 5 to 8. Comparisons of data for relapse versus relapse with vibration groups are shown in Figures 9 to 12. Overall, the mean first molar movement was significantly higher in the control group compared to the other three groups as expected. Nevertheless, when comparing relapse group to relapse with vibration groups, no statistical significance was detected.

DISCUSSION

The aim of our study was to determine whether there is a difference between the amount of relapse after OTM and that observed when the first molar is subject to low-magnitude mechanical vibration at different frequencies in a mouse model. We chose this investigation since there has been a great disparity in the reported findings regarding the effects of vibration both on OTM and relapse. Research on decreasing the rate of relapse via non-fixed retention appliances is scarce, with few studies available [14, 25, 40-44]. Furthermore, since relapse requires OTM, even confounding results have been seen both on a macroscopic and microscopic level in OTM models. Some of the reasons such discrepancies exist are the vast differences in research protocols applied, frequencies or methods of vibration utilized, differing or even un-reported force levels applied in each scenario, and major differences in the various animal models tested in each study. Nevertheless, our objective was to pursue evidence for biological

trends towards an increase in anabolic bone formation, decrease in the rate of tooth movement, decrease in the resorptive rate of osteoclasts, and decrease in root resorption.

In our relapse model, after orthodontic tooth movement, the molar is then free of any orthodontic force and receive low magnitude vibration periodically in daily intervals. The quality and characteristics of bone at our region of interest helps to determine if cyclical loading force may impede the rate of relapse exerted by natural periodontal and soft tissue factors. The ability of bone to adapt to loading forces was described originally by Wilhelm Roux in 1885 and has been known as Wolff's Law [69]. According to this law, when bone is subject to loading forces, the bone will adapt and increase in strength to resist that load. Further studies in jumping rats have also confirmed that there is an anabolic effect observed when increasing the number of jumps per day, which plateaus after a set amount of loading [70]. Compressive and intermittent loads were also applied in avian models, and an increase in bone formation was observed [71]. Rubin et al. have additionally confirmed that bone mineral content and trabecular pattern increase in sheep after 20-50 Hz daily cyclical loading, as well as in humans after specifically 30 Hz of intermittent daily cyclical loading. [46, 72]. Therefore, we also expected to see an anabolic effect in bone formation in our groups.

As our results indicate, there is an increasing trend in TD when comparing the relapse group to the relapse with vibration groups (Figure 10); the TD increases slightly from Relapse to Relapse + 10 Hz, and again from Relapse + 10 Hz to Relapse + 30 Hz. Although this was statistically not significant and very small in percentage (close to 1% gain in TD overall in Relapse + 30 Hz group), the trend suggests anabolic character and follows previous trends in research. While Rubin et al. saw increases in the bone mineral density, which includes a combined density of soft tissue and bone in the region of interest often representative of trabecular bone, our results are

more specific to calcified bone. TD is a density measurement restricted to calcified bone representative of cortical bone excluding soft tissue [46, 72, 73]. In addition, BVF represents the amount of mineralized bone within the volume of the region of interest. Although BVF followed normalized distribution in our models, the results did not demonstrate a significant difference between the relapse groups (Figure 9). Our results further indicate an interesting trend in BVF; the BVF decreased very slightly (0.16%) from Relapse to Relapse + 10 Hz group, and then notably increased from the Relapse to Relapse + 30 Hz model (over 4.8%). The Relapse + 10 Hz group follows trends in BVF of OTM with spring force load and cyclical loading in mouse vibration groups (Dobie and Assefnia, pending publication), showing decreases in mineral density that would help in accelerating OTM. However, in our sample, the difference between BVF of the Relapse and Relapse + 10 Hz groups is negligible. The Relapse + 30 Hz group, though not statistically significant, strongly suggests that the 30 Hz cyclical load may have anabolic effects in increasing mineral density at the region of interest. As mentioned previously, the Relapse + 30 Hz has the most amount of both TD and BVF in our experimental sample. Similar findings were reported in an OTM model with 30 Hz vibration by Kalajzic et al., although not statistically significant, with slight increases in TD and BVF in the 30 Hz vibration groups [53]. Therefore, if the quality of bone is the determinant factor in decreasing the rate of relapse, 30 Hz vibration at alternate daily intervals demonstrates the most potential for increasing cortical bone and decreasing relapse, and requires further investigation within larger samples to obtain statistically significant results.

While our study with relapse and vibration postulates an anabolic response in bone, contradicting results have been reported via OTM models subjected to mechanical vibration in different animal models. Studies in guinea pigs with spring coil and samarium cobalt magnets placed in a pulse

electromagnetic field that provides vibration have demonstrated increases in the rate of OTM [48]. The same groups that demonstrated the above have also demonstrated increased OTM with Neodymium-Iron-Boron magnets and sentalloy closed coil springs with rats [74]. Other sources have also suggested similar findings utilizing different loading techniques and vibration protocols in rats [50], which suggest a catabolic response to bone formation when OTM is combined with constant spring loading as well as intermittent vibration. The AcceleDent^{TM} device with 30 Hz vibration was used in human studies without controls and a 3mm per month OTM in the maxilla and a 2.1mm OTM per month in the mandible was reported when compared with the accepted norm of approximately 1mm per month often seen clinically [52]. However, these studies did not follow the same protocol, and the frequencies of mechanical vibration were different throughout each experiment. The major difference between these studies and our model is that they actively studied OTM while we are considering relapse rates. Our relapse study is one of the first reported dental relapse models in mice, and bears resemblance to another relapse model in rats [25]. In order to relate our model to OTM vibration studies available, the trend in M1-M2 distance needs to be considered, although deemed not statistically significant in our experimental model. In rat spring-loaded studies, the M1-M2 distance increased since OTM was the primary measure. In our sample, since relapse is the primary measure, M1-M2 distance needs to decrease if OTM has been increased, representative of catabolic bone activity. While observing the M1-M2 distance, we noted a clear (over 50%) decrease in M1-M2 distance between the Relapse and Relapse + 10 Hz group, suggestive of increasing catabolic activity. This increase in catabolic activity is also confirmed with the slight decrease in MRV observed in our results for Relapse + 10 Hz when compared to Relapse only group. However, the Relapse + 30 Hz group demonstrates a contradictory increase in M1-M2 distance of 50% compared to the

Relapse group, which is highly suggestive of anabolic bone activity, and is similar to other research findings of Rubin et al. and Kalajzic et al. for this frequency of vibration [46, 53, 72]. Interestingly, when comparing our Relapse + 30 Hz to the Relapse group, we see a slight decrease in MRV, though the MRV for Relpase + 30 Hz is still larger than Relapse + 10 Hz. However, when comparing the MRV to the Controls, we see that the differences are minimal, and the Relapse group has even a slight increase contrary to expectation. Assuming anabolic character of bone with vibration, differences may be due to having more bone turnover at the site due to bone remodeling after OTM, which would recruit osteoclasts leading to increased root resorption. Studies on root resorption have shown that with different types of vibration, root resorption may decrease or remain the same without significant changes [50, 75]. Studies on osteoclasts with vibration have shown that vibration can cause an increase, a decrease, or even have no effect on osteoclast numbers [50, 76, 77]. Changes in MRV in our sample demonstrated negligible changes in root resorption. However, evaluation of changes in osteoclast numbers was not performed as part of this thesis, and will be covered in future research on our saved sample specimens.

While our results seem to correlate with some studies, they also contradict many others. Since the differences between the Relapse group and Relapse with vibration groups are not statistically significant, we cannot draw major implications from the trends observed in our data. Nevertheless, this study has demonstrated important outcomes that help to drive further research in Orthodontics. After the emergence of vibration devices in the market without clear scientific evidence to support industry claims, our research requires further notice. In order to observe statistical significance, our sample sizes need to increase to find conclusive results. The further role of osteoclasts needs to be elucidated in resorption and remodeling rate at the region of

interest. Currently, researchers at the University of Connecticut are analyzing our samples for osteoclast labeling. Also, other mediators up-regulated or down-regulated due to vibration need to be evaluated in the relapse model, which would require detailed studies involving microarrays after induction of vibration at different timepoints. After reviewing that 30 Hz may induce trends towards anabolic bone formation and decrease of relapse in our model, the dosage of vibration and duration needs to be experimentally determined to find the ideal dosage and duration necessary for best outcomes. Another factor is also the onset of release of secondary mediators after vibration. Since our vibration protocol required vibration only one day prior to release of the molar from constant spring loading, other vibration onset times need to be investigated to pinpoint the best response in anabolic activity. Following completion of more experiments, we may be able to define better protocols in reducing orthodontic relapse in animal models, and later in humans. This will brighten the future of orthodontics and help to sustain optimal occlusion and esthetics after orthodontic treatment.

CONCLUSIONS

Our research design is the first relapse study performed on a mouse model to date. We were able to determine statistically significant differences between first molar distances amongst our control and experimental samples, while other outcomes such as bone volume fraction, tissue density, and mesial root volume did not show any significant changes. However, we did not find any statistical significance between the relapse and relapse with vibration groups, and were unable to indicate a positive statistically significant effect of vibration on retention. This may have been due to our small sample sizes or limited frequencies and dosages of vibration tested. Nonetheless, there was a clear trend toward 30 Hz vibration suggesting anabolic bone formation during relapse, as well as a decrease in tooth movement following 30 Hz intermittent cyclical

loading. Our results correspond to results in previous tooth movement publications [53]. Further study of molecular mechanisms involved in OTM and relapse combined with larger sample sizes and different dosages, modes, and frequencies of vibration are necessary to shed light on providing better means of orthodontic retention in the future.

TABLES AND FIGURES

Figure 1. Application of Orthodontic Force: Ni-Ti spring appliance in the mouth consisting of a Ni-Ti coil spring attached to the maxillary right first molar (left yellow arrow) and both central incisors (right yellow arrow) via two separate segments of 0.004" annealed stainless-steel (SS) ligature wire. To prevent any dislodging, the wire around the incisors is secured using a composite resin. Mouth is being held open with college pliers. Lips are retracted with a custom mouth-prop fabricated from 0.017" x 0.025" TMA wire utilized during application of vibration (see below).

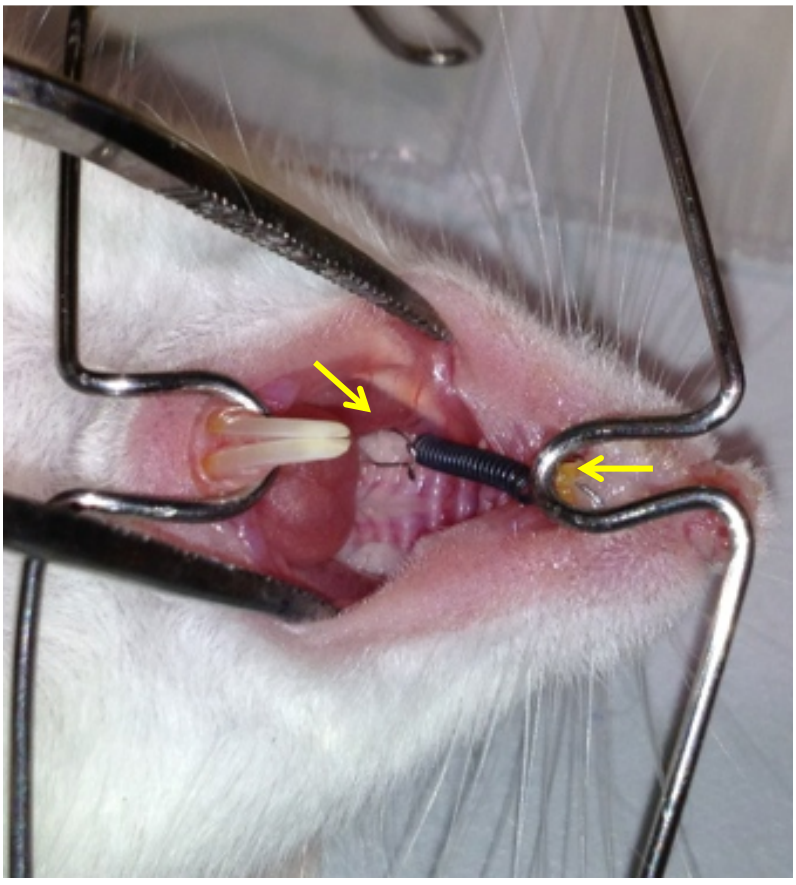


Figure 2. Bose Electromechanical Actuator: Bose model of the feedback-loop controlled, electromechanical actuator (Model 3230, Bose/EnduraTec, Minnetonka, MN) utilized to apply unilateral mechanical vibration to the occlusal surface of the mouse maxillary right first molar along the long axis of the tooth.

Figure 3. Application of Mechanical Vibration: tip of electromechanical actuator (Model 3230, Bose/EnduraTec, Minnetonka, MN) is touching the occlusal surface of the maxillary right first molar (yellow arrow). Mouth is being held open with a custom mouth-prop fabricated from 0.017" x *0.025"* TMA wire utilized during application of vibration.

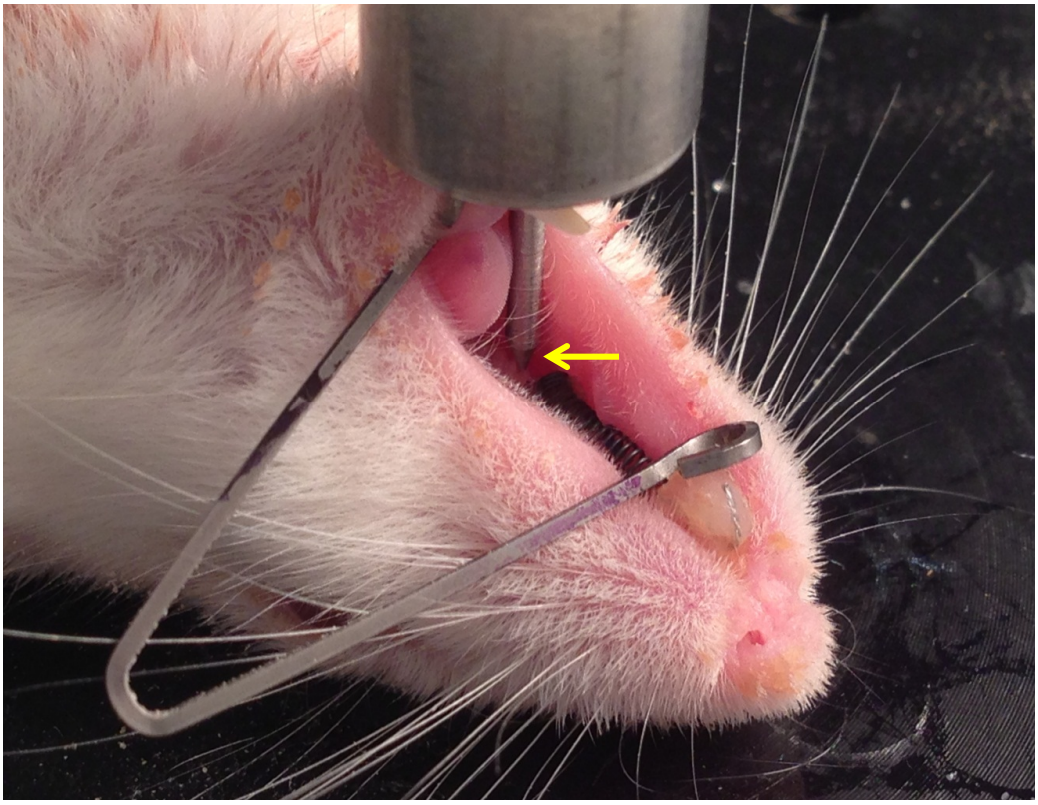


Figure 4. Saggital view of Region of Interest (ROI) for micro-CT measurements of BVF and TD. The mouse first molar is the large molar on the left side. Mesial is oriented toward left side, and distal is oriented toward right side of this image.

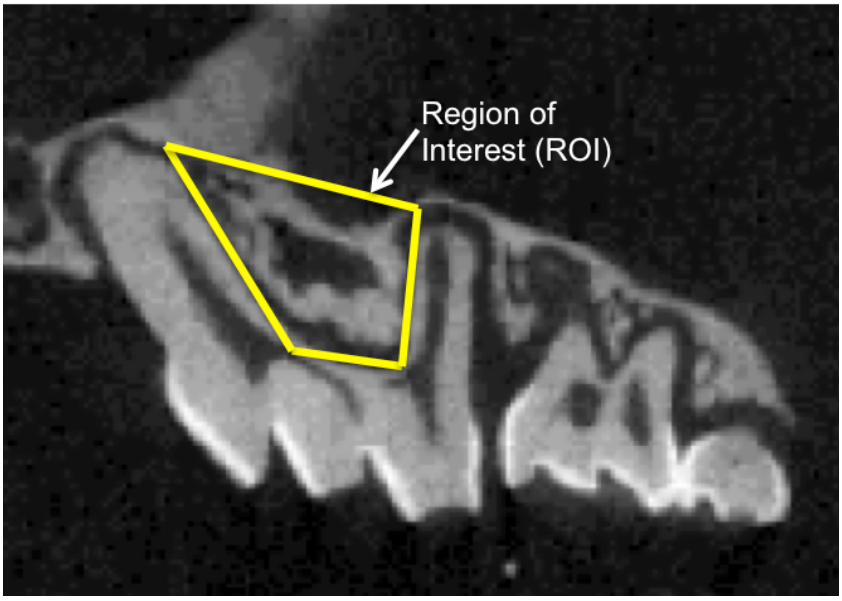


Table 1. Test for normality distribution of data: Kolmogorov-Smirnov Test was modified to serve as a goodness of fit test [78]. In testing for normality of the distribution, samples are standardized and compared with a standard normal distribution. A p-value< 0.008 concludes that the two groups were sampled from populations with different distributions. Bone volume fraction (BVF) and mesial root volume (MRV) are normally distributed, while tissue density (TD) and M1-M2 distance are not normally distributed.

One-Sample Kolmogorov-Smirnov Test

		BVF	TD	M1-M2 Distance	MRV
N		34	34	34	34
Normal Parameters[a,b]	Mean	80.521%	1124.683685	.04974	.22694
	Std. Deviation	5.7311%	91.1443781	.047544	.031075
Most Extreme Differences	Absolute	.077	.298	.179	.112
	Positive	.067	.175	.179	.112
	Negative	-.077	-.298	-.148	-.053
Test Statistic		.077	.298	.179	.112
Asymp. Sig. (2-tailed) p-value		**.200[c,d]**	**<.0001[c]**	**.007[c]**	**.200[c,d]**

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

d. This is a lower bound of the true significance.

Table 2. Outcomes by group.

Measure		BVF	TD	M1-M2 Distance	(MRV)
Control Group					
Mean		80.63%	1159.85926	0.1155	0.22048
Std. Deviation		6.47%	32.541784	0.039896	0.033088
Minimum		66.70%	1108.9318	0.054	0.181
Maximum		86.50%	1209.9741	0.174	0.278
Percentiles	25	77.52%	1135.7072	0.075	0.1925
	50	82.57%	1156.0503	0.126	0.21765
	75	85.41%	1187.0206	0.138	0.2483
Relapse Group					
Mean		79.04%	1037.65408	0.0308	0.23815
Std. Deviation		6.34%	129.54363	0.028974	0.030496
Minimum		68.40%	856.0641	0	0.187
Maximum		86.10%	1179.4683	0.09	0.279
Percentiles	25	73.11%	914.383175	0	0.21373
	50	82.12%	1048.23735	0.0275	0.24305
	75	83.94%	1166.3521	0.04975	0.2647
Relapse Group + 10 Hz					
Mean		78.88%	1158.72884	0.012	0.21925
Std. Deviation		4.66%	20.8430233	0.02222	0.040238
Minimum		73.70%	1132.7018	0	0.153
Maximum		89.10%	1201.0795	0.048	0.294
Percentiles	25	75.44%	1146.70393	0	0.19873
	50	78.66%	1153.45735	0	0.2209
	75	79.63%	1170.9036	0.036	0.2384
Relapse Group + 30 Hz					
Mean		83.92%	1164.24996	0.04538	0.22708
Std. Deviation		4.48%	27.2985222	0.019683	0.018649
Minimum		78.20%	1122.6259	0	0.209
Maximum		88.60%	1207.055	0.06	0.254
Percentiles	25	80.04%	1147.5552	0.0415	0.21157
	50	84.06%	1159.8722	0.052	0.2204
	75	88.18%	1189.75265	0.05775	0.24688

Table 3. Difference amongst control and experimental groups per outcome measurement.

Measurement	**Test**	**p-Value**
Bone volume fraction	One-way ANOVA	0.252
Tissue density	Kruskal-Wallis Test	0.243
M1-M2 Distance	Kruskal-Wallis Test	<0.0001*
Mesial root volume	One-way ANOVA	0.564

*Statistically Significant ($p<0.008$)

Table 4. Pair-wise comparisons for BVF.

Pair-wise Comparison	p-value
Control versus Relapse	0.99
Control versus Relapse + 10 Hz	0.99
Control versus Relapse + 30 Hz	0.99
Relapse versus Relapse + 10 Hz	0.99
Relapse versus Relapse + 30 Hz	0.46
Relapse + 30 Hz versus Relapse + 10 Hz	0.50

*Statistically significant ($p<0.008$)

Table 5. Pair-wise comparisons for tissue density.

Pair-wise Comparison	p-value
Control versus Relapse	0.10
Control versus Relapse + 10 Hz	0.96
Control versus Relapse + 30 Hz	0.79
Relapse versus Relapse + 10 Hz	0.20
Relapse versus Relapse + 30 Hz	0.10
Relapse + 10 Hz versus Relapse + 30 Hz	0.72

*Statistically significant ($p<0.008$)

Table 6. Pair-wise comparisons for M1-M2 Distance.

Pair-wise Comparison	p-value
Control versus Relapse	<0.0001*
Control versus Relapse + 10 Hz	<0.0001*
Control versus Relapse + 30 Hz	0.002*
Relapse versus Relapse + 10 Hz	0.17
Relapse versus Relapse + 30 Hz	0.12
Relapse + 10 Hz versus Relapse + 30 Hz	0.02

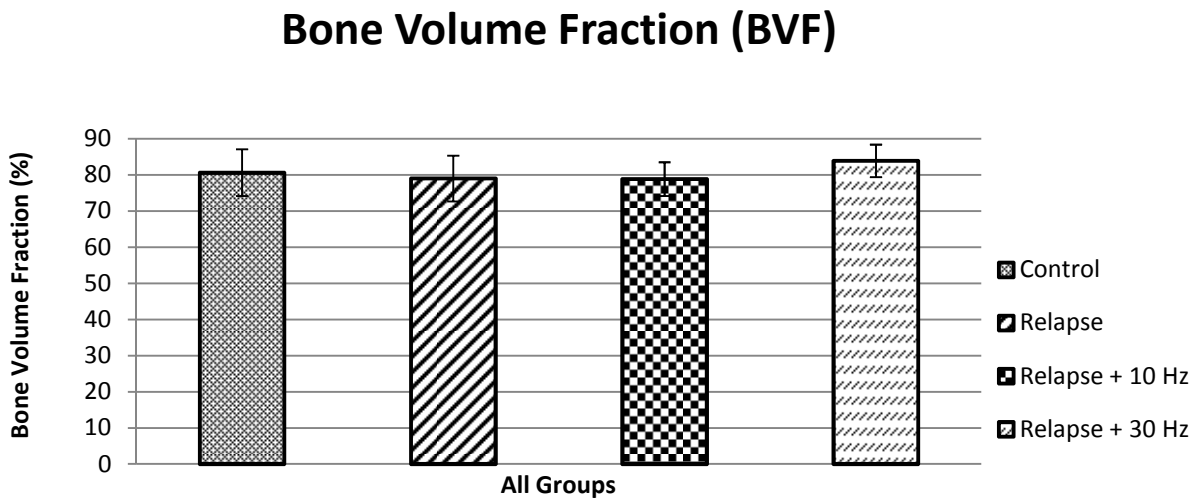
*Statistically significant ($p<0.008$)

Table 7. Pair-wise Comparisons for mesial root volume.

Pair-wise Comparison	p-value
Control versus Relapse	0.99
Control versus Relapse + 10 Hz	0.99
Control versus Relapse + 30 Hz	0.99
Relapse versus Relapse + 10 Hz	0.99
Relapse versus Relapse + 30 Hz	0.99
Relapse + 10 Hz versus Relapse + 30 Hz	0.99

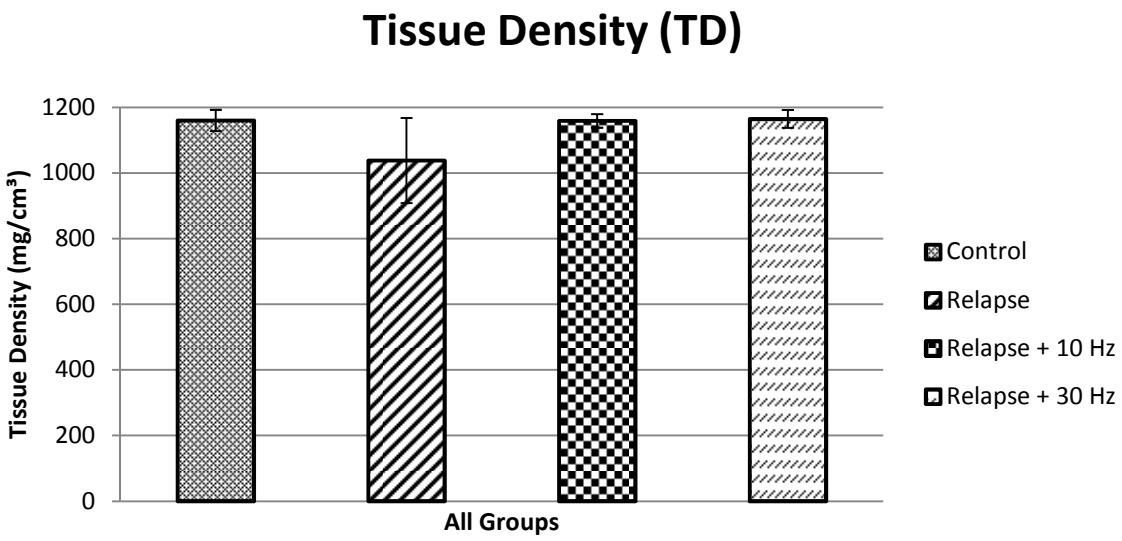
*Statistically significant ($p<0.008$)

Figure 5. Bone volume fraction data across all groups.



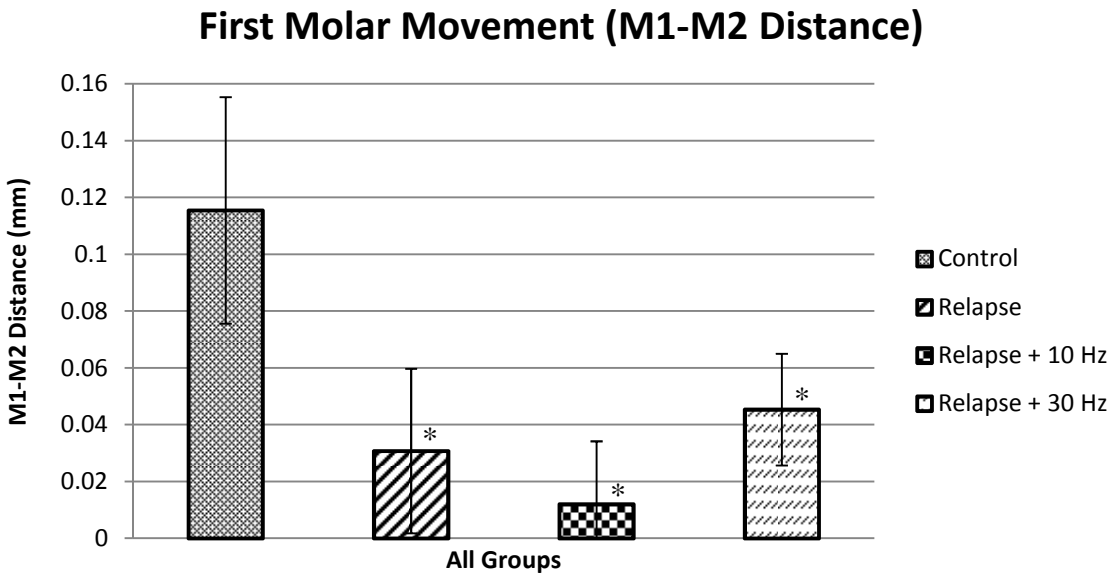
*Statistically significant ($p<0.008$)

Figure 6. Tissue density data across all groups.



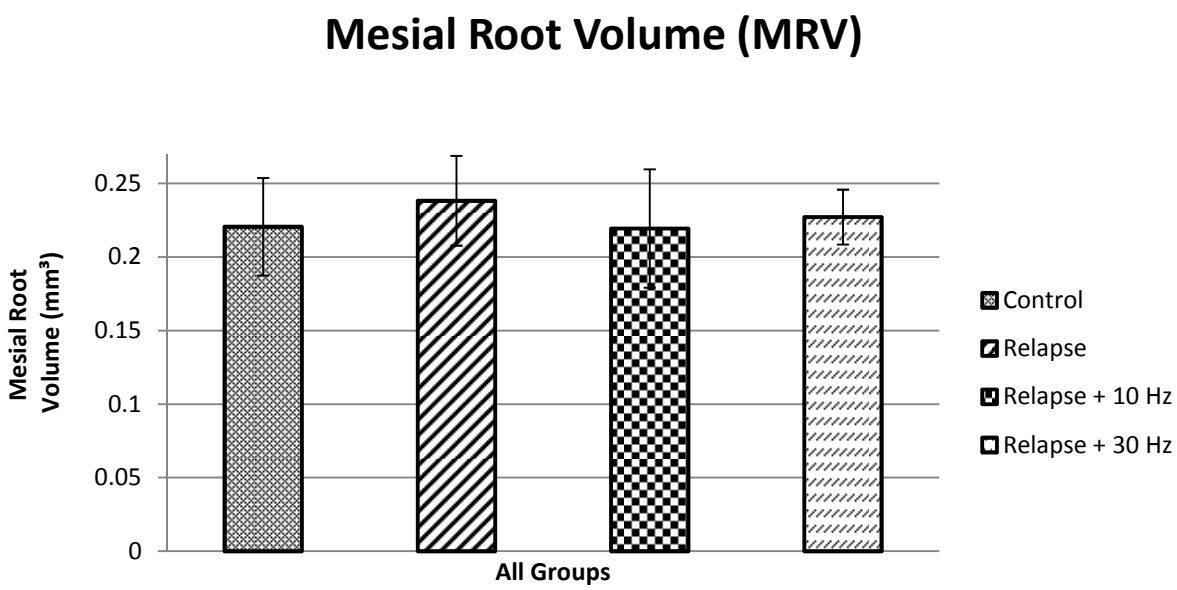
*Statistically significant ($p<0.008$)

Figure 7. First molar movement (M1-M2 Distance) data across all groups. Statistical significant movement was observed between controls versus all experimental groups.



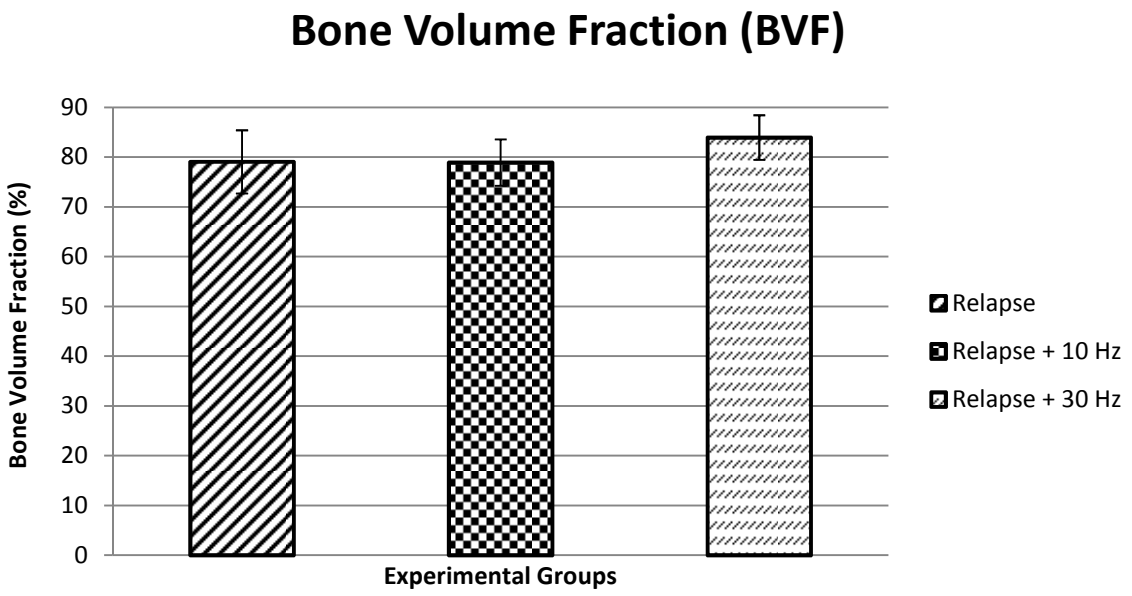
*Statistically significant ($p<0.008$)

Figure 8. Mesial root volume data across all groups.



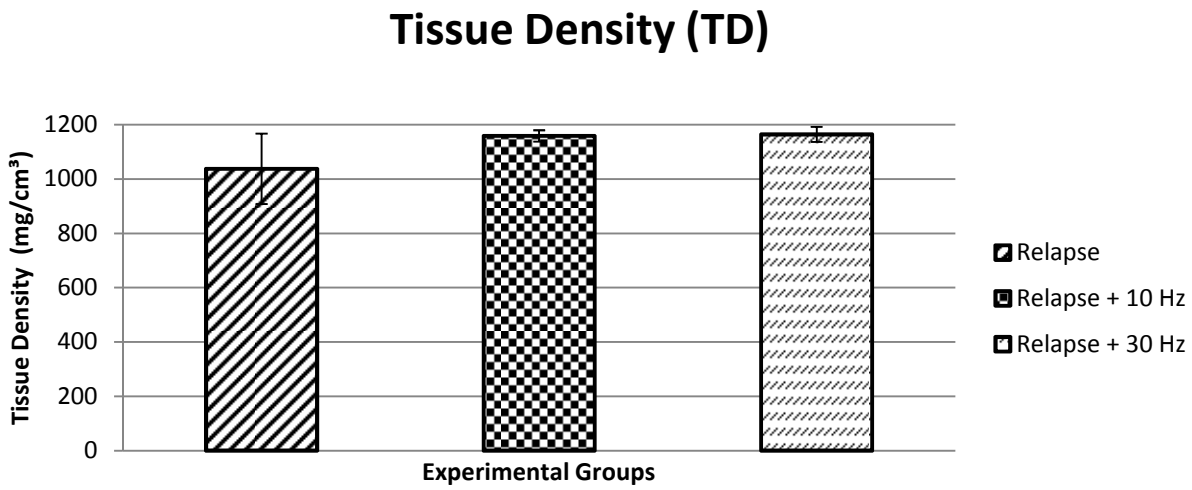
*Statistically significant ($p<0.008$)

Figure 9. Bone volume fraction across experimental groups.



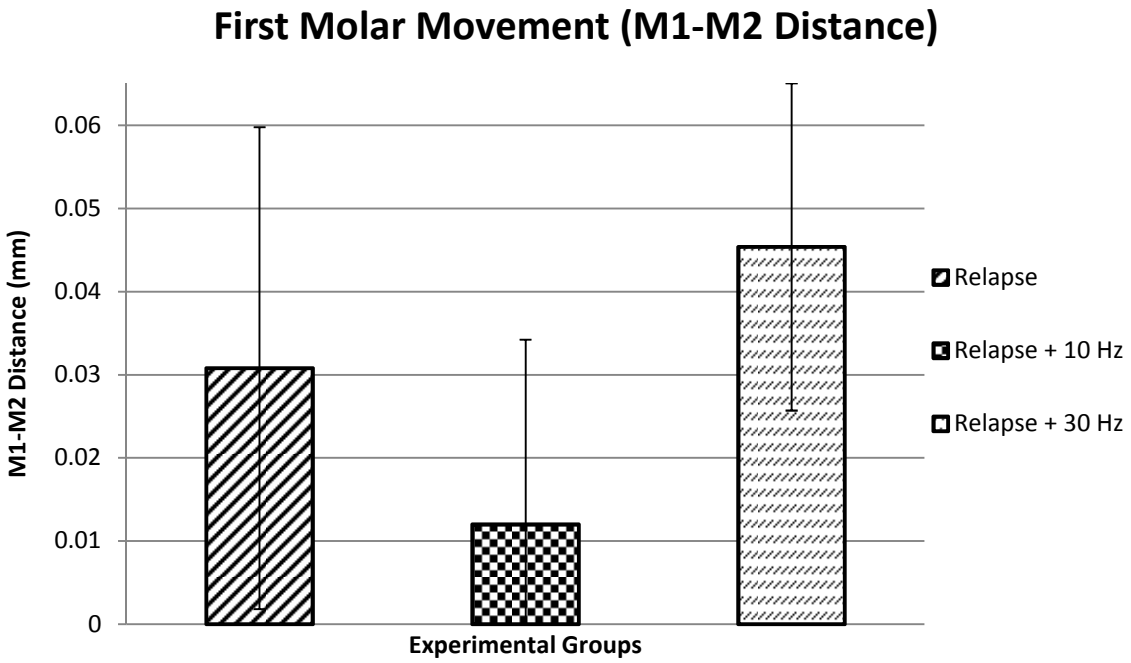
*Statistically significant ($p<0.008$)

Figure 10. Tissue density data across experimental groups.



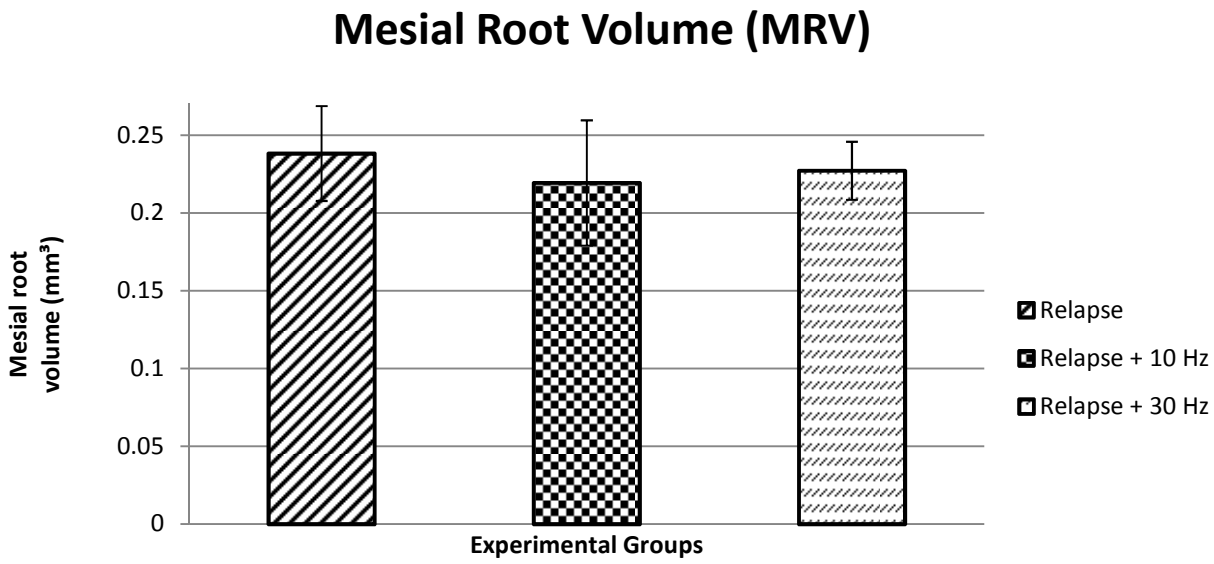
*Statistically significant ($p<0.008$)

Figure 11. First molar movement (M1-M2 Distance) data across experimental groups.



*Statistically significant ($p<0.008$)

Figure 12. Mesial root volume data across experimental groups.



*Statistically significant ($p<0.008$)

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