Biomarkers of orthodontic tooth movement with fixed appliances and vibration appliance therapy: A randomized clinical trial

Principal Investigator
Flavio Uribe DDS, MDentSc

Division of Orthodontics
Department of Craniofacial Sciences
University of Connecticut Health Center

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1) **Title**

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2) **Background**

Orthodontic clinical treatment ranges between 21-27 months for nonextraction treatment and 25-35 months for extraction treatment[1]. Reducing clinic treatment time has a number of major advantages, including reducing the risk of caries[2] and root resorption[3]. In addition, one may expect that extended treatment times impact patient compliance as well contribute to negative perceptions that ultimately deter individuals from seeking orthodontic treatment. There is therefore great interest to identify novel methods which modulate orthodontic tooth movement and increase the speed and efficiency of treatment.

The application of orthodontic force results in remodeling of the dental pulp, periodontal ligament, alveolar bone and surrounding soft tissues.[4] A number of factors have been shown to be increased with the application of orthodontic force and to activate osteoclasts and osteoblasts, the chief cells of bone remodeling. Regulation of the Receptor Activator of Nuclear Factor Kappa B/ Osteoprotegrerin (RANKL/OPG) axis has been studied both *in vitro* and in animal models of tooth movement. This system appears to play a central role in the tightly coupled process of bone remodeling. RANKL is expressed in osteoblasts and is responsible for the recruitment, differentiation, and survival of osteoclasts. OPG is a soluble decoy receptor for RANKL which is both expressed in osteoclasts and functions to block RANKL-mediated osteoclast activation in a negative feedback loop maintaining homeostasis between bone formation and resorption. Numerous orthodontic studies in animal models have highlighted the role of the RANKL/OPG system [5-7] and more recently, temporal changes during orthodontic tooth movement have been measured in a humans.[8]

Other pro-inflammatory cytokines, interleukins (ILs) and matrix metalloproteinases (MMPs) are also known to be activated in response to orthodontic force. Tumor Necrosis Factor alpha (TNFα) and interleukins have been shown to directly and indirectly induce osteoclastogenesis. TNFα[9], IL-1[10,11], IL-6[12,13], IL-8[14] and IL-17[15] have been shown to be significantly elevated in response to orthodontic force. Matrix metalloproteinases (MMPs) are important biomarkers of tissue breakdown and are involved in collagen breakdown during bone remodeling. A number of MMPs have been shown to have increased expression during orthodontic tooth movement.[16,17] MMP-9

has been shown to be increased in gingival crevicular fluid in response to orthodontic force.[18-21] MMP-13 has also been shown to be highly expressed in the Periodontal Ligament (PDL) and adjacent alveolar bone during tooth movement.[22,23]

A number of approaches have been employed to enhance the biological mechanisms involved in tooth movement including pharmaceutical therapies using parathyroid hormone (PTH)[24], thyroxine[25], Vitamin D3 [1,25 $(OH)_2D_3$][26], and prostaglandins[27]. While these agents can increase tooth movement, they also have adverse effects including local pain, severe root resorption and drug-induced side effects[28,29]. An added physical approaches, such as vibration, may modulate the local microenvironment and alter the expression of key biological factors involved in tooth movement without adverse effects.

Vibration and Orthodontic Tooth Movement

Studies examining the effect of cyclical loading have shown potential for an acceleration of tooth movement when vibration is added to fixed forces. At the cellular level, vibration has been shown to enhance the osteogenic potential of PDL stem cells, driving increased expression of bone formation factors like type I collagen, Runx2 and Osterix. These stem cells also show a frequency-dependent increase in alkaline phosphatase and osteocalcin expression in the range of 40Hz to 120Hz[30]. Resonance vibration of 60Hz when applied intermittently has been shown to accelerate tooth movement in rats, possibly through an increase in RANKL expression within the PDL[31]. In addition, pulsed electromagnetic field vibration has been shown to accelerate tooth movement in rats when used in conjunction with fixed appliance therapy[32].

At the clinical level, a randomized controlled clinical trial in humans by Pavlin and colleagues showed a two-fold increase in the speed of alignment of the mandibular dentition and a 38% reduction in the time of space closure with the AcceleDent vibration appliance used twice daily at 10 minute intervals (unpublished data). Using the same protocol of vibration another study showed that, there was no significant difference in the speed of alignment, with similar reductions in irregularity at 10 weeks compared to subjects not undergoing vibration therapy[33]. Based on the limited evidence available, it is clear that further clinical and translational studies are required to determine the potential for this appliance to accelerate orthodontic tooth movement and better understand the biologic processes that may be involved.

Vibration Treatment- Bone Remodeling Biomarker Measurement

Identifying factors that are differentially expressed in response to mechanical force and further modulated by additive vibration could help to:

1. define the complex pathways involved in and
2. provide information that would be useful for further accelerating orthodontic tooth movement.

Changes at the molecular and cellular levels ideally should be examined in real time to better understand the temporal changes after force activation. For osteoclasts, markers of bone resorption are currently available. One primary indicator of bone resorption are the breakdown products of type-I collagen which subsequently results in an increase in byproducts such as C-telopeptides of type 1 collagen (CTX) that are detectable in the serum[34], urine[35] and saliva[36]. In addition, osteoblast activity can be measured in real time by evaluating markers of bone formation such as osteocalcin (OC)[37,38] and bone specific alkaline phosphatase (ALP).

Evaluating the expression of specific markers of bone remodeling in orthodontic patients using a vibration appliance may well aid our understanding the molecular pathways involved in accelerating tooth movement. The research methods used should have a high level of sensitivity for the targets of interest and be obtained by a minimally invasive method for patients, especially considering the target clinical age group . Utilizing saliva as a means of evaluating biomarkers is a newly emerging field in oral diagnostics and is showing great promise.[39] In orthodontics, the use of salivary biomarkers is in its early stages but showing great potential. To date, only a few studies have evaluated the salivary changes in the expression of different bone remodeling factors[8,40,41] during orthodontic tooth movement. Understanding the complex molecular pathways involved in orthodontic tooth movement as well as how specific factors are differentially expressed when combined with vibration treatment could be valuable to help identify future targets of treatment.

Vibration Treatment- Pain and Quality of Life During Orthodontic Treatment

In addition to a potential role in accelerating tooth movement, vibration may help reduce the orthodontic pain experienced in the early stages following force activation. A few studies have examined the role of vibration on pain. One study showed that in dental patients receiving local anesthesia, vibration applied extra-orally near the injection site resulted in reduced pain parameters and perceptions of pain.[42] Temporal Mandibular Dysfunction (TMD) pain has been shown to be reduced in response to vibration therapy.[43] Only one orthodontic study has examined

the role of vibration treatment in orthodontic patient, not showing a significant difference in pain experiences during the first week of treatment.[33] It could be useful to understand how the pain experienced by the orthodontic patient changes over a longer period of time and over subsequent visits (15-17 weeks) to better understand the role of vibration treatment in modulating orthodontic pain.

Studies have shown that the oral health quality of life (OHQoL) is impacted by orthodontic treatment. After orthodontic treatment, studies have shown a significant improvement in OHQoL compared to baseline measures.[44,45] However, during orthodontic treatment, OHQoL reduces significantly, as has been shown in younger patients.[46] Motivation measures related to self-empowerment and participation in treatment have been shown to improve the overall OHQoL in schoolchildren.[47] Limited studies are available regarding the OHQoL during orthodontic treatment and further studies are needed to determine which factors contribute to improving the overall experience for patients successfully completing orthodontic nonextraction treatment with tooth movement .

Study Rationale

Currently, orthodontic treatment typically lasts approximately 2 years. There are a number of advantages for reducing the duration of treatment, including reducing the potential risk of caries[2], root resorption[3] and for minimizing patient “burn out” from prolonged treatment.

While some evidence does exist that vibration may accelerate the speed of tooth movement, the biological mechanism is still unknown. Identification of specific factors involved in tooth movement that are further stimulated by vibration would help to understand the mechanisms involved as well as discover possible biologic targets which could be utilized or modified to maximize the benefits of vibration treatment.

The purpose of this study is to identify novel biological factors that are expressed in patients undergoing orthodontic tooth movement in conjunction with vibration appliance therapy.

Outcome Assessments

- *Primary outcome:* Changes in the expression of salivary biomarkers of bone remodeling
- *Secondary outcomes:* Changes in 1) tooth mobility, 2) change in alignment 3) changes in pain and OHQoL

Hypotheses, aims and objectives

Scientific Hypotheses

1) The expression of biological markers of bone remodeling are increased in combined vibration-fixed appliance treatment.
2) The degree of tooth mobility is increased in patients undergoing combined vibration-fixed appliance treatment compared to orthodontic treatment alone.
3) The percent change in alignment is greater in patients undergoing combined vibration-fixed appliance treatment compared to controls during the first 6 months of treatment.
4) The orthodontic treatment pain will be reduced in patients undergoing combined treatment with a vibration appliance compared to controls.

General Objectives

Few studies exist examining the effect of vibration appliances on the rate of orthodontic tooth movement in patients. Furthermore, there is a great need to better understand the biological mechanisms by which vibration treatment may enhance tooth movement. *The primary objective of this study is to identify potential factors involved in bone remodeling which may be regulated during fixed appliance treatment in conjunction with vibration therapy.*

Specific Aims

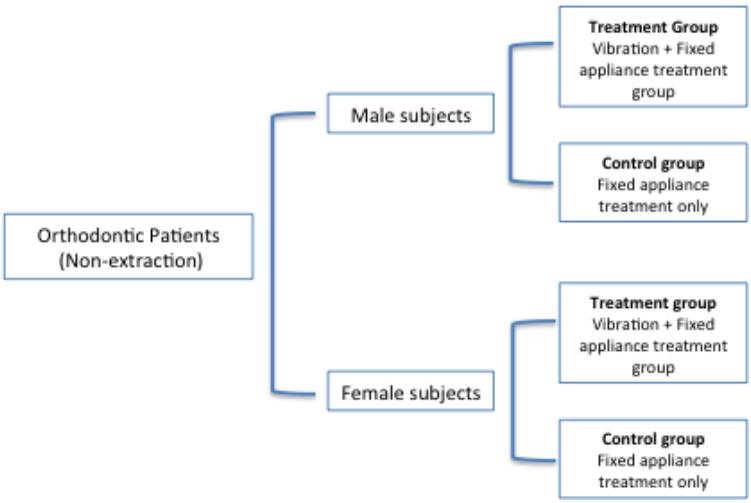
1) To determine if combined vibration-fixed appliance treatment alters the expression of specific bone remodeling factors in saliva compared to fixed orthodontic treatment alone.
2) To further elucidate the role of vibration treatment on the degree of tooth mobility during fixed appliance treatment compared to control.
3) To determine if combined vibration-fixed appliance treatment increases the speed of orthodontic tooth movement during the alignment phase of treatment.
4) To evaluate the role of vibration treatment in the control of pain in patients undergoing orthodontic treatment.

Study Design and Screening Procedures

Study Design: Randomized controlled clinical trial:

N=40 @ UCHC Orthodontic Clinic, enrolled pre-bonding

10 Randomized to each Arm



Screening & Recruitment Procedures

Approximately 600 patients begin treatment every year in the University of Connecticut Orthodontic Clinic. The pilot project aim is to recruit a total of 40 patients, with 20 male and 20 female subjects, equally divided between control and vibration groups (above).. Based on the projected starts, we anticipate meeting the recruitment goals during the first 12 months of the study.

Prospective subjects will be screened for this study after completing all the usual initial screening procedures for patients in the University of Connecticut Orthodontic Clinic with their appointed primary orthodontic provider. The primary orthodontic provider will determine if the adult patient is likely to qualify (pre-screen) for the study based on the inclusion/exclusion criteria. The initial clinical indicators of eligibility evaluated during the pre-screen are a healthy patient undergoing non-extraction treatment with good oral hygiene. Male and female subjects of all ethnicities will be included if between the ages of 15-35 years of age.

If the prospective adult patient meets the initial criteria, the orthodontic provider will notify the study coordinator and PI to screen the patient's existing dental records, including screening forms, models and/or radiographs for confirmation of likely eligibility for participation in the study.

When children ages 15-17 are considered for pre-screen, the provider will first obtain permission from one parent and then from the patient prospect prior to determining eligibility from the screening of existing records. If the child passes screening, the parent would be first approached by the provider about interest in participation, before also approaching the child and making a referral to the PI or Study Coordinator.

Enrollment

After the primary orthodontic provider pre-screens and identifies an interested potential subject for the study coordinator, the study coordinator will meet to discuss the study with the subject at the next appointed visit, typically the Orthodontic records appointment, at least 1 week prior to band bonding. Prior to conducting consent, the study coordinator will confirm the patient's is eligibility for the study based on review of the patient's clinical chart and the following inclusion/exclusion criteria:

Inclusion Criteria	Exclusion Criteria
Healthy, non-smoker with no systemic medical conditions and no routine medications	Patients that require extractions as part of the orthodontic plan
15 to 35 years of age at the time of bonding	Smoking or excessive alcohol consumption
Non-extraction treatment plan or no extractions required in the first 6 months of treatment	Patients with edentulous areas
At least 5mm of crowding in the mandibular arch	Evidence of periodontal disease (any pocket depths more than 4mm)
Full-complement dentition 1st molar to 1st molar	Use of anti-inflammatory drugs within 2 days of bonding
Good oral hygiene	Active oral lesions (ulcerations, sores, mucositis, etc.)
	Uncontrolled diabetes
	Dentofacial deformities (cleft palate, hemifacial microsomia, etc.)
	Subjects routinely taking any of the following medications: Corticosteroids (including for asthma) Bisphosphonates Anti-inflammatories Nicotine Patch Estrogen Opioids Growth Hormone Relaxin Anti-coagulants
	Disease that could affect bone metabolism: Parathyroid or thyroid dysfunction Osteoporosis, osteomalacia Vitamin D deficiency Fibrous dysplasia Paget's Disease Multiple Myeloma Osteogenesis Imperfecta History of Bone Metastasis
	Patients taking medications such as bisphosphonates, corticosteroids or any anti-inflammatory drug

If the patient meets the above criteria, the study coordinator will further discuss the details of the study and obtain consent from the patient and/or parent (if the patient is under the age of 18)- in a private manner and after all questions having been answered. Patients must be enrolled into the study prior to the routinely scheduled bonding of fixed appliances by their provider. Instructions will be given to the patient/parent prior to the first study visit (T0), including to not take any analgesic medications prior to the first appointment.

Study Procedures

I. *Standardized Orthodontic Treatment Protocol*

The orthodontic clinic treatment protocol will be standardized to minimize variability in the sequence of treatment. All subjects will be bonded with 0.022"X0.028" brackets passive self-ligating brackets with maximal band tension (MBT) prescription from 2nd premolar to 2nd premolar in both arches and bonded tubes on the 1st molars at the baseline (T0) visit. Each subject will have a 0.014" copper nickel titanium (NiTi) archwire placed at the initial visit. At T2, the next archwire in the sequence (0.014"X0.025" copper NiTi) will be placed.

Subjects will be seen at normally appointed visits every 4-6 weeks. The primary orthodontic provider will direct the alignment and leveling phase of treatment and proceed through the wire sequence as indicated. No specific procedure/timeframe will be assigned to placement of each archwire. Providers are expected to proceed to the next archwire when it can be placed comfortably based on the degree of alignment achieved.

In the event of bracket breakages, the subject is to be seen within 7 days to have the bracket rebonded, in standard care as well as the study. The primary orthodontic provider will reposition the bracket in the ideal position and continue with alignment using the study archwires. If the subject does not present with 7 days of the breakage, he/she will be disqualified from the study.

II. *Randomization Procedures*

Once recruited to the study and after baseline measures are taken at T0 by the study coordinator, the randomization will be performed. Since study groups will be subdivided by gender, separate randomization will be performed for males and females. Block randomization will be used. Based on gender, 20 opaque envelopes will be generated containing the group allocation (10 for the vibration study group, 10 for the non-vibration control group). At the time of randomization, the

subject will choose an envelope and disclose the group assignment. For subjects allocated to the vibration group, the study coordinator will provide detailed instructions as to how to operate the appliance and will instruct those subjects to use it for 20 minutes per day.

Timetable of Study Assessments

Assessment/ Visit	Screen	Baseline T0	Visit T1	Visit T2	Visit T3
Week	2d-to -6w	0	5-6	10-12	15-17
Consent	X				
Inclusion Criteria/ Confirm	X	X			
Alginate Impression		X	X	X	X
Randomization *		X			
Braces applied /bonded		X			
Vibration Appliance instructions**		X			
Saliva Collection		X	X	X	X
Clinical Data		X	X	X	X
Periotest		X	X	X	X
Pain Diary*** & OHIP 14		X	X	X	X***
Model Analysis		X	X	X	X
Record Adverse Effects		X	X	X	X

*Randomization before bonding
**For vibration group only- SC initially provides device and manufacturer's instructions.
*** Diary provided @ Baseline & visit T1, T1 to be collected on next visit.

III. *Data Collection Procedures (See Study Timetable)*

On the day of bonding (T0), prior to the placement of any appliances, baseline unstimulated whole saliva will be collected and Periotest will performed on selected teeth in the mandibular arch. In addition, alginate model impressions will be taken. These procedures will be described in more detail in the next sections. Once baseline measures have been taken, the PI will perform the randomization and notify the study coordinator as to which group the subject is assigned to ("vibration treatment" or "no vibration"). The patients allocated to the vibration treatment group

will be provided with the AcceleDent appliance and instructed to use the 20 minutes per day according to the manufacturer's instructions.

After fixed appliances are placed and tooth movement begins, subjects will proceed with standard orthodontic treatment with or without vibration treatment based on allocation. Collection of saliva samples, Periotest and alginate impressions will be taken at T1 (5-6 weeks post-T0), T2 (10-12 weeks post-T0) and T3 (15-17 weeks post-T0). Subjects will be seen between noon and 5pm to help minimize possible confounding of salivary markers by circadian rhythms.

Salivary Biomarkers

Collection of unstimulated whole saliva will be performed using the same methods described by Navazesh and Kumar[48]:

"The patient is advised to refrain from intake of any food or beverage (water exempted) one hour before the test session. Smoking, chewing gum and intake of coffee also are prohibited during this hour. The subject is advised to rinse his or her mouth several times with deionized (distilled) water and then to relax for five minutes. The patient is then told the following: "I will first obtain measures of saliva flow while you are at rest. This means that before and during the collection you should make every effort to minimize movement, particularly movements of your mouth. To begin a collection trial, I will ask you to swallow to void the mouth of saliva. Then you should lean your head forward over the test tube and funnel" (demonstrate). "Keep your mouth slightly open and allow saliva to drain into the tube. Keep your eyes open. At the end of the collection period, I will ask you to collect any remaining saliva in your mouth and spit it into the test tube. This movement should be done very quickly and should be done in the same manner from trial to trial. This is very important. Do you understand the procedures?"

When you start a trial, tell the subject:

1. Swallow to begin a trial (begin timing).

2. Make as little movement as possible. Do not swallow, and keep your eyes open during collection periods.

3. At the conclusion of the trial, collect the remaining saliva and spit it out."

Unstimulated whole saliva will be collected by passive drooling into sterile centrifuge tubes on ice for 15 minutes or once 2-10mL of saliva is collected at baseline and each visit, whichever occurs

first[49]. Once saliva is collected, samples will be kept on ice and supplemented with proteinase inhibitor. Samples will then be centrifuged for 10 minutes at 3000 rpm to remove cellular debris and supernatants will be collected and stored at -80°C until they are ready to be analyzed for biomarkers[50].

Biomarkers will be evaluated by ELISA assay by a direct sandwich method using a standard protocol. Primary antibodies to selected factors will be pre-coated onto 96 well dishes. Sample product or standards will be incubated on the coated plates and a secondary conjugated antibody will be used to detect binding using chemiluminescence. Selected target biomarker antibodies include ALP, RANKL/TRANCE, OPG, Osteocalcin (marker of bone formation), MMP8, MMP13, TNF α, IL1a, IL1b, IL3, IL6, IL11 and IL18. Human antibodies to these targets will be obtained from R&D Systems, Inc. In addition, salivary C-terminal telopeptide of type I collagen (CTX) (an indicator of bone resorption) will be analyzed by ELISA assay using a RatLaps kit (Immunodiagnosis System, Inc.)

Periotest Measurement Procedures

This measurement is obtained at baseline and each visit The degree of tooth mobility will be used using a Periotest device (Siemens AG, Bensheim, Germany) on the central incisors, canines and 2nd premolars in both mandibular quadrants as previously described by Liou *et al*.[51] The archwire will be removed and the Periotest measurements will be taken in triplicate, with means recorded. The Periotest handpiece will be positioned such that the tip is 2mm from the labial surface and perpendicular to the tooth surface for measurements. The examiner will locate an area on the labial surface that has sufficient space for the tip to contact the tooth surface and be able to take consistent measurements. Detectable Periotest values (PTV) range from -8.0 to +50, with data values correlated, as below, to the degree of mobility based on Miller's index[52,53]:

PTV Measure	Indication
-8.0 to +9.0	No movement distinguishable
+10.0 to +19.0	First distinguishable sign of mobility
+20.0 to +29.0	Crown deviates within 1mm of normal position
+30.0 to +50.0	Mobility is easily noticeable

Dental Model Analysis

Two blinded clinical evaluators will assess the rate of alignment of the lower incisors for both groups. Analyses of dental casts will be done on the mandibular cast using a method based on Little's irregularity index. Little's irregularity index was developed to assess amount of anterior tooth displacement from canine to canine (see Cast Irregularity Data Collection Form). Contact displacement will be measured in millimeters around the arch from canine to canine. Irregularity will be measured on models from each of the four time points and be evaluated based on percentage change over the 6-month evaluation period.

Orthodontic Pain Assessment

Subjects will be given a pain diary on the baseline (T0), visits T1 & T2 to record the level of orthodontic pain each evening for the first 7 days after each study visit. The degree of pain will be assessed using a Visual Analog Scale (VAS) ranging from 1 (least pain) to 10 (highest pain)[54]. In addition, patients will report if the pain is constant or only present on biting/chewing[55]. The subjects will return the completed diary to the next visit to be collected by the study coordinator and kept in the study record.

Oral Health Quality of Life (OHQoL)

To assess the impact of the vibration appliance on the overall oral health quality of life of the subject, subjects will be given an Oral Health Impact Profile (OHIP-14) questionnaire[56]. The OHIP-14 consists of 14 questions divided into specific dimensions including functional limitation, physical pain, psychological discomfort, physical disability, psychological and social disability and overall life handicap from orthodontic treatment with or without the vibration appliance. The OHIP-14 (exhibit below) will be administered at each study visit and the profile changes will be compared across time points in data analyses.

OHIP-14 Questionnaire:

Dimension	Question	Weight
Functional Limitation	Have you had trouble *pronouncing any words* because of problems with your teeth or mouth?	0.51
	Have you felt that your *sense of taste* has worsened because of problems with your teeth or mouth?	0.49
Physical Pain	Have you had *painful aching* in your mouth?	0.34
	Have you found it *uncomfortable to eat any foods* because of problems with your teeth or mouth?	0.66
Psychological Discomfort	Have you been *self-conscious* because of your teeth or mouth?	0.45
	Have you *felt tense* because of problems with your teeth or mouth?	0.55
Physical Disability	Has your *diet been unsatisfactory* because of problems with your teeth or mouth?	0.52
	Have you had to *interrupt meals* because of problems with your teeth or mouth?	0.48
Psychological Disability	Have you found it *difficult to relax* because of problems with your teeth or mouth?	0.60
	Have you been a bit *embarrassed because* of problems with your teeth or mouth	0.40
Social Disability	Have you been a bit *irritable with other people* because of problems with your teeth or mouth?	0.62
	Have you had *difficulty doing your usual jobs* because of problems with your teeth or mouth?	0.38
Handicap	Have you felt that life in general was *less satisfying* because of problems with your teeth or mouth?	0.59
	Have you been *totally unable to function* because of problems with your teeth or mouth?	0.41

Each question is answered on a 5-point scale:

0=never

1=hardly ever

2=occasionally

3=fairly often

4=very often

Individual scores are multiplied by the weights to give a subscale score for each category.

IV. *Sample size and justification*

Few studies exist evaluating salivary biomarkers during orthodontic tooth movement. Our primary outcome is changes in the expression of specific biomarkers of cellular activation and bone remodeling. No RCTs are currently available to predict treatment effects on biomarker expression. This study will serve as a pilot project with 40 patients total in 4 groups divided by gender and vibration/no-vibration treatment arms.

We estimate 600 patients in the target age range beginning orthodontic treatment at the University of Connecticut Orthodontic Clinic each year. We anticipate meeting our recruitment goal for this study.

V. *Subject Participation*

The timing of visit activity is planned to coincide with normally scheduled clinic provider/patient visits. Typically, treatment for non-extraction cases last from 12-30 months in the Orthodontic clinic using fixed appliances. However, the subjects will only be evaluated during the first 6 months of their clinical appliance treatment for the study purpose and data. Providers will follow patients every 5-6 weeks and the study coordinator will ensure/confirm the patients for study appointments per Timetable, pg. 9. After completion of the observation and data collection phase (up to 17 weeks), the patients will continue to proceed with treatment as directed by the their primary orthodontic provider. Data collection will occur at normally scheduled visits with no additional appointments specific to the study.

In the event that the PI determines that a subject is not suited to continue in the study (eg. the subject shows consistent failure to keep appointed visits, consistent breakage of appliances, patient safety considerations, etc.) he/she will be withdrawn by the PI from the study and no further data will be collected. Specifically, poor compliance with attending appointments during the allotted time frame for each study visit will result in subjects being disqualified from the study. For example, the follow-up time frame for T1 is 5-6 weeks after T0 (baseline). Therefore if the patient misses an appointment and cannot or does not reschedule to attend his/her appoint during the allotted time from (by 6 weeks after T0), then he/she will be disqualified from the study. Also, in the event of bracket breakages (as stated earlier), the subject is to be seen within 7 days to have the bracket rebounded. The primary orthodontic provider will reposition the bracket in the ideal position and continue with alignment using the study archwires. If the subject does not present with 7 days of the breakage, he/she will be disqualified from the study.
The data collected to that point will be utilized in analyses. The patient's treatment will continue with the primary orthodontic provider consistent with the standard of care.

VI. *Statistical/Data Analysis*

Intra and inter-rater reliability of the irregularity measurements will be assessed using the T0 and T3 models evaluated by the two blinded evaluators one week apart for all patients. A Cronbach alpha analysis will be used to assess reliability of the measurements. A single calibrated evaluator

(the study coordinator) will collect all tooth mobility measurements with the Periotest and means will be evaluated using non-parametric statistical analyses.

A Wilcoxon Rank Sum test for independent samples will be used to assess differences between groups for all the continuous variables with an α=0.05 for irregularity index changes-Periotest measurements, VAS &OHIP-14 measures.

Salivary biomarker expression will be analyzed by ANOVA to assess differences between the four study groups at each time point. The study coordinator and principal investigator will perform statistical analyses.

VII. *Timetable Table Timeline of Performance Goals:*

Activity	Year 1-2014				Year 2-2015			
IRB, Staff training & preparation	X							
Subject Recruitment &Enrollment	X	X	X					
Final Assessments/Endpoints (17 weeks)						X		
Data Entry & Cleaning						X	X	X
Data analyses							X	X
Report writing								X

Expected Start Date: 2/1/2014

General Time Table

January 2014

Submit IRB for full board review

February 2014

Enrollment begins

Begin data collection for T0 time point in recruited subjects

August 2014

Initially recruited subjects complete study to final endpoint (T4)

June 2015

All consented subjects will have completed study

Data collection complete (N=20)

July 2015

Data analyses conducted

August 2015

Manuscript Preparation

- ***Expected Completion Date:*** August 2015

5) **Budget**

Patients will be responsible for covering the cost of orthodontic treatment in the University of Connecticut Orthodontics Clinic (either directly or with insurance). However, a deduction of $400.00 on the standard orthodontic fees will be given to every patient enrolling in the research. The participants will receive this incentive in the form of $100.00 at each research appointment completed until the maximum benefit is reached. If a participant is not compliant with the study protocol, he/she will be dismissed from the study and will not receive any further compensation. The same orthodontic procedures will be used for all subjects in this study regardless of study group.

Please see the attached budget detailing the study-specific costs.

6) **Dissemination**

The results of this research will be published in a peer-reviewed dental journal. In addition, this work is likely to be presented at national/international meetings.

References

1. Buschang PH CP, Ruso S. . Accelerating tooth movement with corticotomies: is it possible and desirable? . Semin Orthod 2012;18:286-294.
2. Julien KC, Buschang PH, Campbell PM. Prevalence of white spot lesion formation during orthodontic treatment. The Angle Orthodontist 2013;83:641-647.
3. Pandis N, Nasika M, Polychronopoulou A, Eliades T. External apical root resorption in patients treated with conventional and self-ligating brackets. American Journal of Orthodontics and Dentofacial Orthopedics 2008;134:646-651.
4. Krishnan V, Davidovitch Z. On a path to unfolding the biological mechanisms of orthodontic tooth movement. Journal of dental research 2009;88:597-608.
5. Kanzaki H, Chiba M, Arai K, Takahashi I, Haruyama N, Nishimura M et al. Local RANKL gene transfer to the periodontal tissue accelerates orthodontic tooth movement. Gene Ther 2006;13:678-685.
6. Kim T, Handa A, Iida J, Yoshida S. RANKL expression in rat periodontal ligament subjected to a continuous orthodontic force. Arch Oral Biol 2007;52:244-250.
7. Shiotani A, Shibasaki Y, Sasaki T. Localization of receptor activator of NFkappaB ligand, RANKL, in periodontal tissues during experimental movement of rat molars. J Electron Microsc (Tokyo) 2001;50:365-369.
8. Florez-Moreno GA, Isaza-Guzman DM, Tobon-Arroyave SI. Time-related changes in salivary levels of the osteotropic factors sRANKL and OPG through orthodontic tooth movement. Am J Orthod Dentofacial Orthop 2013;143:92-100.
9. Andrade I, Jr., Silva TA, Silva GA, Teixeira AL, Teixeira MM. The role of tumor necrosis factor receptor type 1 in orthodontic tooth movement. Journal of dental research 2007;86:1089-1094.
10. Lee TY, Lee KJ, Baik HS. Expression of IL-1beta, MMP-9 and TIMP-1 on the pressure side of gingiva under orthodontic loading. The Angle Orthodontist 2009;79:733-739.
11. Basaran G, Ozer T, Kaya FA, Kaplan A, Hamamci O. Interleukine-1beta and tumor necrosis factor-alpha levels in the human gingival sulcus during orthodontic treatment. The Angle Orthodontist 2006;76:830-836.
12. Madureira DF, Taddei Sde A, Abreu MH, Pretti H, Lages EM, da Silva TA. Kinetics of interleukin-6 and chemokine ligands 2 and 3 expression of periodontal tissues during orthodontic tooth movement. American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics 2012;142:494-500.
13. Jacobs C, Walter C, Ziebart T, Grimm S, Meila D, Krieger E et al. Induction of IL-6 and MMP-8 in human periodontal fibroblasts by static tensile strain. Clinical oral investigations 2013.
14. Asano M, Yamaguchi M, Nakajima R, Fujita S, Utsunomiya T, Yamamoto H et al. IL-8 and MCP-1 induced by excessive orthodontic force mediates odontoclastogenesis in periodontal tissues. Oral diseases 2011;17:489-498.
15. Compressive force stimulates the gene expression of IL-17s and their receptors in MC3T3-E1 cells. Connective Tissue Research 2010;51:359-369.
16. Canavarro C, Teles RP, Capelli Junior J. Matrix metalloproteinases -1, -2, -3, -7, -8, -12, and -13 in gingival crevicular fluid during orthodontic tooth movement: a longitudinal randomized split-mouth study. European journal of orthodontics 2012.
17. Lisboa RA, Andrade MV, Cunha-Melo JR. Toll-like receptor activation and mechanical force stimulation promote the secretion of matrix metalloproteinases 1, 3 and 10 of human periodontal fibroblasts via p38, JNK and NF-kB. Archives of oral biology 2013;58:731-739.
18. Grant M, Wilson J, Rock P, Chapple I. Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement. European journal of orthodontics 2012.
19. Lee TY, Lee KJ, Baik HS. Expression of IL-1beta, MMP-9 and TIMP-1 on the pressure side of gingiva under orthodontic loading. The Angle Orthodontist 2009;79:733-739.

20. Bildt MM, Bloemen M, Kuijpers-Jagtman AM, Von den Hoff JW. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in gingival crevicular fluid during orthodontic tooth movement. European journal of orthodontics 2009;31:529-535.
21. Capelli J, Jr., Kantarci A, Haffajee A, Teles RP, Fidel R, Jr., Figueredo CM. Matrix metalloproteinases and chemokines in the gingival crevicular fluid during orthodontic tooth movement. European journal of orthodontics 2011;33:705-711.
22. Leonardi R, Talic NF, Loreto C. MMP-13 (collagenase 3) immunolocalisation during initial orthodontic tooth movement in rats. Acta histochemica 2007;109:215-220.
23. Takahashi I, Nishimura M, Onodera K, Bae JW, Mitani H, Okazaki M et al. Expression of MMP-8 and MMP-13 genes in the periodontal ligament during tooth movement in rats. Journal of dental research 2003;82:646-651.
24. Lee WC. Experimental study of the effect of prostaglandin administration on tooth movement--with particular emphasis on the relationship to the method of PGE1 administration. Am J Orthod Dentofacial Orthop 1990;98:231-241.
25. Verna C, Dalstra M, Melsen B. The rate and the type of orthodontic tooth movement is influenced by bone turnover in a rat model. Eur J Orthod 2000;22:343-352.
26. Collins MK, Sinclair PM. The local use of vitamin D to increase the rate of orthodontic tooth movement. Am J Orthod Dentofacial Orthop 1988;94:278-284.
27. Yamasaki K, Shibata Y, Fukuhara T. The effect of prostaglandins on experimental tooth movement in monkeys (Macaca fuscata). J Dent Res 1982;61:1444-1446.
28. Sekhavat AR, Mousavizadeh K, Pakshir HR, Aslani FS. Effect of misoprostol, a prostaglandin E1 analog, on orthodontic tooth movement in rats. American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics 2002;122:542-547.
29. Brudvik P, Rygh P. Root resorption after local injection of prostaglandin E2 during experimental tooth movement. European journal of orthodontics 1991;13:255-263.
30. Zhang C, Li J, Zhang L, Zhou Y, Hou W, Quan H et al. Effects of mechanical vibration on proliferation and osteogenic differentiation of human periodontal ligament stem cells. Archives of oral biology 2012;57:1395-1407.
31. Nishimura M, Chiba M, Ohashi T, Sato M, Shimizu Y, Igarashi K et al. Periodontal tissue activation by vibration: intermittent stimulation by resonance vibration accelerates experimental tooth movement in rats. American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics 2008;133:572-583.
32. Darendeliler MA, Zea A, Shen G, Zoellner H. Effects of pulsed electromagnetic field vibration on tooth movement induced by magnetic and mechanical forces: a preliminary study. Australian dental journal 2007;52:282-287.
33. Miles P, Smith H, Weyant R, Rinchuse DJ. The effects of a vibrational appliance on tooth movement and patient discomfort: a prospective randomised clinical trial. Australian orthodontic journal 2012;28:213-218.
34. Henriksen K, Andersen JR, Riis BJ, Mehta N, Tavakkol R, Alexandersen P et al. Evaluation of the efficacy, safety and pharmacokinetic profile of oral recombinant human parathyroid hormone [rhPTH(1-31)NH(2)] in postmenopausal women with osteoporosis. Bone 2013;53:160-166.
35. Nagase S, Hashimoto Y, Small M, Ohyama M, Kuwayama T, Deacon S. Serum and urine bone resorption markers and pharmacokinetics of the cathepsin K inhibitor ONO-5334 after ascending single doses in post menopausal women. British journal of clinical pharmacology 2012;74:959-970.
36. Salivary biomarkers: Relationship between oxidative stress and alveolar bone loss in chronic periodontitis. Acta odontologica Scandinavica;0:1-6.

37. Bullon P, Berta Goberna, Juan M. Guerrero, Juan J. Segura, Ramon Perez-Cano, and Angel Martinez-Sahuquillo.
Serum, Saliva, and Gingival Crevicular Fluid Osteocalcin: Their Relation to Periodontal Status and Bone Mineral Density in Postmenopausal Women. Journal of Periodontology 2005;76:513-519.
38. Vs K, K P, Ramesh M, Venkatesan V. The association of serum osteocalcin with the bone mineral density in post menopausal women. Journal of clinical and diagnostic research : JCDR 2013;7:814-816.
39. Tatarakis N, Kinney JS, Inglehart M, Braun TM, Shelburne C, Lang NP et al. Clinical, microbiological, and salivary biomarker profiles of dental implant patients with type 2 diabetes. Clin Oral Implants Res 2013.
40. Ellias MF, Zainal Ariffin SH, Karsani SA, Abdul Rahman M, Senafi S, Megat Abdul Wahab R. Proteomic analysis of saliva identifies potential biomarkers for orthodontic tooth movement. ScientificWorldJournal 2012;2012:647240.
41. Florez-Moreno GA, Marin-Restrepo LM, Isaza-Guzman DM, Tobon-Arroyave SI. Screening for salivary levels of deoxypyridinoline and bone-specific alkaline phosphatase during orthodontic tooth movement: a pilot study. Eur J Orthod 2013;35:361-368.
42. Nanitsos E, Vartuli R, Forte A, Dennison PJ, Peck CC. The effect of vibration on pain during local anaesthesia injections. Australian dental journal 2009;54:94-100.
43. Roy EA, Hollins M, Maixner W. Reduction of TMD pain by high-frequency vibration: a spatial and temporal analysis. Pain 2003;101:267-274.
44. Feu D, Miguel JA, Celeste RK, Oliveira BH. Effect of orthodontic treatment on oral health-related quality of life. The Angle Orthodontist 2013;83:892-898.
45. Palomares NB, Celeste RK, Oliveira BH, Miguel JA. How does orthodontic treatment affect young adults' oral health-related quality of life? American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics 2012;141:751-758.
46. Brosens V, Ghijselings I, Lemiere J, Fieuws S, Clijmans M, Willems G. Changes in oral health-related quality of life reports in children during orthodontic treatment and the possible role of self-esteem: a follow-up study. European journal of orthodontics 2013.
47. Nammontri O, Robinson PG, Baker SR. Enhancing oral health via sense of coherence: a cluster-randomized trial. Journal of dental research 2013;92:26-31.
48. Navazesh M, Kumar SK, University of Southern California School of D. Measuring salivary flow: challenges and opportunities. J Am Dent Assoc 2008;139 Suppl:35S-40S.
49. Tatarakis N, Kinney JS, Inglehart M, Braun TM, Shelburne C, Lang NP et al. Clinical, microbiological, and salivary biomarker profiles of dental implant patients with type 2 diabetes. Clinical oral implants research 2013.
50. Miricescu D, Totan A, Calenic B, Mocanu B, Didilescu A, Mohora M et al. Salivary biomarkers: Relationship between oxidative stress and alveolar bone loss in chronic periodontitis. Acta odontologica Scandinavica 2013.
51. Liou EJ, Chen PH, Wang YC, Yu CC, Huang CS, Chen YR. Surgery-first accelerated orthognathic surgery: postoperative rapid orthodontic tooth movement. Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons 2011;69:781-785.
52. Schulte W, d'Hoedt B, Lukas D, Maunz M, Steppeler M. Periotest for measuring periodontal characteristics--correlation with periodontal bone loss. Journal of periodontal research 1992;27:184-190.
53. Jonsson A, Malmgren O, Levander E. Long-term follow-up of tooth mobility in maxillary incisors with orthodontically induced apical root resorption. European journal of orthodontics 2007;29:482-487.

54. Doshi-Mehta G, Bhad-Patil WA. Efficacy of low-intensity laser therapy in reducing treatment time and orthodontic pain: a clinical investigation. American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics 2012;141:289-297.
55. Tecco S, D'Attilio M, Tete S, Festa F. Prevalence and type of pain during conventional and self-ligating orthodontic treatment. European journal of orthodontics 2009;31:380-384.
56. Slade GD. Derivation and validation of a short-form oral health impact profile. Community dentistry and oral epidemiology 1997;25:284-290.