7-6-2016

Biomarkers of Orthodontic Tooth Movement with Fixed Appliances and Vibration Device: A Randomized Clinical Trial

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A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Dental Sciences

At the

University of Connecticut

2016

APPROVAL PAGE

Master of Dental Sciences Thesis

Biomarkers of Orthodontic Tooth Movement with Fixed Appliances and Vibration Device: A Randomized Clinical Trial

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University of Connecticut
2016

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Chapter I: Introduction

A- Background

On average, comprehensive orthodontic treatments last approximately 21-27 months in non-extraction cases and 25-35 months when extractions are considered in the treatment plan. [1] Longer treatment time has been associated with multiple detrimental effects such as white spot lesions [2], root resorption [3], gingival inflammation [4] and dental caries. Additionally, increased treatment time often leads to the exhaustion of the patient's compliance. It is then in the patient's and in the clinician's interest to identify methods to increase the speed and efficiency of treatment. It has been estimated that normal tooth movement occurs at a rate of 0.8-1.2 mm/month. [1] As yet, research has focused on three main modalities to enhance the rate of tooth movement: pharmacological, surgical and mechanical approaches. Local or systemic administration of biological factors such as parathyroid hormone (PTH), thyroxine, Vitamin D3 and prostaglandins have been investigated in various experiments and have been found to increase the velocity of tooth movement. However, using these approaches has also shown some systemic adverse effects, such as pain and severe root resorption. Some surgical techniques such as osteotomy, corticotomy, corticision and piezocision have shown possible increases of orthodontic tooth movement by taking advantage of the Regional Acceleratory Phenomenon. However, due to its invasive nature, patients are less inclined to consent to this method. Furthermore, numerous studies have demonstrated the short term effect of this RAP phenomenon, lasting on average only 2-4 weeks. Finally, the application of mechanical vibration to the dentition has also been hypothesized to increase the rate of tooth movement by affecting the expression of key biological factors involved in bone remodeling.

The application of orthodontic forces results in remodeling of the alveolar bone through activity of important cells such as osteoblasts and osteoclasts. A number of key factors have been shown to activate osteoclastogenesis, the RANK/RANKL/OPG signaling pathway being one of them. RANKL is a molecular biomarker secreted by osteoblasts, responsible for the recruitment, differentiation and survival of osteoclasts. The binding of RANKL with RANK (expressed at the surface of the osteoclast) induces the differentiation of the immature osteoclasts into functional cells. Meanwhile, osteoprotegerin (OPG) is also produced by the osteoblasts and acts as a soluble receptor for RANKL, inhibiting the terminal stages of osteoclast differentiation. [5] It serves as a negative feedback maintaining homeostasis between bone formation and resorption. The role of the OPG/RANKL system in bone remodeling has been illustrated in several studies performed on animals [6] [7] [8] and recently on humans during orthodontic treatment. [9]

1- Vibration and Orthodontic Tooth Movement

A patient's primary concern with fixed orthodontic appliances is the time required for treatment. Since the development of a vibrating mouthpiece device for orthodontic purposes in 1982 by Kurz, application of external vibrational force has spawned some interest in academic literature. [10] Animal studies examining the effect of vibration have shown potential for an acceleration of tooth movement, stimulating the inflammation process by possibly altering the periodontal apparatus or by creating osteogenic effects. [11] In a study performed on rats, Nishimura and colleagues demonstrated that the application of resonance vibration at 60 Hz, accelerated orthodontic tooth movement via increased expression of

RANKL in the periodontal ligament. [12] Additionally, pulsed electromagnetic field vibration delivered eight hours per day on Wistar rats has also shown significantly accelerated tooth movement. [13] Recently, several studies have demonstrated that the application of mechanical vibration with low-magnitude and high frequency can enhance bone remodeling, prevent bone loss, and improve bone healing in animals and humans. [14] However, the process by which this outcome is seen is not clearly understood. In a study where vibration was applied to stem cells that had been isolated from extracted premolars, the collected data demonstrated that mechanical vibration promotes osteogenic differentiation of human periodontal stem cells and increases osteogenesis markers. [14]

At the clinical level, few randomized clinical trials have been published. In 2009, an attempt to reproduce vibration delivery in humans was made by the confection of a novel device named AcceleDent, applying cyclic forces of 25g at a frequency of 30 Hz. In a case series including 14 patients, Kau et al. noticed a rate of tooth movement of 2.1 mm per month in the mandibular arch while 3.0 mm was observed in the maxilla, the majority of the results being measured in terms of reduction of Little's Index scores. They then concluded this rate was statistically significantly faster than the usual 1 mm per month of movement reported in the literature. [15] A randomized clinical trial was then performed by Pavlin et al., assessing the rate of space closure during canine retraction. The results showed an average monthly tooth movement rate of 1.16 mm/month when the AcceleDent appliance was used for 20 minutes daily, corresponding to an increase of 48% in the rate of space closure. [16] Shortly thereafter, another RCT was performed by an Australian group of authors using a slightly different vibration device. Notably, this device called the *Tooth Masseuse* delivered a

vibrational force of higher frequency but lower amplitude than the Acceledent device. Looking at Little's irregularity index, they concluded there was no statistically significant difference between control and experimental groups. [17] In a similar protocol, Bowman et al found an increase rate of leveling and aligning during comprehensive treatment by 30% and 29-40% respectively. [18] Conversely, Woodhouse and colleagues recently conducted a randomized clinical trial which found no evidence that supplemental vibration added to conventional orthodontic treatment, increase the rate of initial tooth movement or reduce the amount of time required to achieve final alignment. [19] Finally, in a recent systematic review in the Cochrane Library, the authors concluded that the available evidence is of very low quality and it is not possible to determine if there is a positive effect of vibration device in conjunction of fixed appliances to accelerate tooth movement. [20] Based on this evidence, this branch of orthodontics is currently still controversial. There is therefore a clear need for well-designed clinical trials in order to determine the actual effect of the application of cyclical forces on the rate of tooth movement.

2- Vibration Treatment- Bone Remodeling Biomarker Measurement

Orthodontic tooth movement results from remodeling of the periodontal ligament and alveolar bone after the inflammatory process has been initiated. [9] Vibrational loading is claimed to stimulate bone remodeling; however, the biological mechanism underlying this effect is not clearly understood. Does it activate the known signaling pathways of tooth movement or does it activate a new one? Identifying factors that are differently expressed when orthodontic force is applied could help our profession to fully understand this complex mechanism and could also guide us toward different target factors in our pursuit of the

acceleration of the rate of tooth movement. An important marker to illustrate the rate of bone turnover is the RANKL/OPG ratio and multiple studies have clearly detected these cytokines during orthodontic tooth movement. [9] [5] More specifically, biological factors can be categorized in relation to their role in bone formation or bone resorption. The former can be measured by evaluating the bone formation markers such as alkaline phosphatase (ALP) [21] and osteocalcin (OC) in saliva, in gingival crevicular fluid and in blood. [22] [23] In a study performed on rats, Hashimoto et al. have shown an increase in the rate of orthodontic tooth movement when OC was injected at the bifurcation of the maxillary first molar. [24] Regarding bone resorption, osteoclast activity can be represented by the breakdown product of type I collagen such as C-terminal telopeptide (CTX).

Early phase of tooth movement involves an acute inflammatory process accompanied by vascular vasodilation, immune cell migration as well as secretion of multiple chemical messengers. Pro-inflammatory cytokines, interleukins and matrix metalloproteinases are also known to be activated in response to orthodontic treatment. Cytokines are active molecules that regulate the inflammatory process. When they bind to a cellular receptor, they can influence diverse biological activities, such as immune function and cellular activation, proliferation and survival. Tumor Necrosis Factor alpha (TNF-α) and interleukins are cytokines that have been shown to be increased with orthodontic force application in rats [25] and humans [26] and to be involved in the induction of osteoclastogenesis. Studies in bone remodeling have indicated that certain interleukins such as IL-1 [27] [28] [26], IL-6 [29] [30], IL-8 [31] and IL-17 [32] are important regulators in the bone remodeling process and thus have shown increased levels during orthodontic force application. For instance, interleukin-

1β, a protein involved in the mediation of inflammation, has been shown to rise in GCF within a short time after the application of pressure. [26] [27] Finally, of the inflammatory mediators that are involved in alveolar bone resorption, matrix metalloproteinases (MMPs) have been implicated in orthodontic tooth movement. They represent a family of proteases that play key roles in collagen breakdown and serve as important biomarkers of bone remodeling. Multiple studies have shown increased expression of certain metalloproteinases during orthodontic treatment. Among these MMPs, increased levels of MMP-9 were found in the gingival crevicular fluid in response to external pressure on teeth. [33] [28] [34] [35] MMP-13 was also highly expressed in the periodontal ligament and alveolar bone early on following the application of an orthodontic force. [36] [37]

Evaluating the expression of different biomarkers of bone remodeling in patients undergoing orthodontic treatment in combination with vibration devices could help us clarify the specific biomechanical pathways engaged when methods of tooth movement acceleration are used. The test chosen to conduct the assessment must have an acute sensitivity to the factors of interest and needs to be relatively minimally invasive in order to have a good acceptance from patients. Multiple methods to assess biological factors have been used in the literature; blood, gingival crevicular fluid and saliva being some examples. Recently, we have noted an increased used of saliva analysis in the oral health field. It is claimed to be a mirror of the body and is used as a diagnostic tool that has many advantages such as its non-invasive nature, its ease of use and the fact that sufficient quantities can be often easily obtained for analysis. [38] It has previously been employed in the detection of caries risk, periodontitis, oral cancer, breast cancer, salivary gland disease, hepatitis, HIV and HCV [39]. In

orthodontics, despite that only few studies have been conducted evaluating saliva for the expression of multiple bone remodeling factors, this newly emerging field shows great promise.

3- *Vibration Treatment- Pain and Quality of Life During Orthodontic Treatment*

In addition to the potential increase in rate of tooth movement, it has been hypothesized that vibration may help in the reduction of dental pain during active orthodontic treatment. Two randomized clinical trials comparing a control group and an experimental group obtained contradictory results: one concluded decreased pain when vibration appliance was used [40] while the other study found no statistically significant difference [17]. Recently, another study including a sham device group incorporated in the design was published and supported the previous Australian conclusion with no significant difference found in the pain level during the week following the placement of fixed appliance and wire insertion [41].

Recent studies have shown that malocclusion is also associated with poor Oral Health Quality of Life (OHQoL) [42]. However, as yet, the literature does not give conclusive evidence on the psychosocial effect of orthodontic treatment. In studies performed on a Brazilian population, they found that patients who received orthodontic treatment (children as much as adults) had significantly better OHQoL after treatment is completed than untreated subjects [43] [42]. However, research [44] has also shown that some patients go through a transitional phase of deterioration of the OHQoL during the active orthodontic phase. [45] Due to limited literature regarding the effect of orthodontic treatment on OHQoL,

further research is needed to assess to psychosocial impact as well as the possible factors that could contribute to improve the overall experience of the patients during treatment.

B- Study Rationale

Currently, orthodontic treatment usually lasts approximately 2 years. There are multiple advantages for reducing the treatment time; decrease risk of root resorption and decalcification [46], maintain good periodontal health as well as minimize patient "burn out" from prolonged treatment.

Some clinical studies using a vibration device in conjunction with fixed appliances have assessed the acceleration of tooth movement, showing some contradictory results. Furthermore, a recent systematic review published in the Cochrane Journal has stipulated a very low level of evidence among these articles [44]. There is thus a clear need of well-designed clinical studies in order to elucidate the clinical effect of vibration.

Additionally, the biological mechanism during acceleration of the rate of tooth movement is still unknown. Identification of specific biomarkers in the saliva that may be stimulated by the use of a vibration device could help the profession to understand the pathways involved and could lead to new biological factors that could be targeted to achieve the goal to reduce orthodontic treatment time.

The aim of this study is to evaluate the effects of vibration on the rate of alignment of the mandibular anterior teeth and to identify biological factors that are expressed with this therapy.

Outcome assessment

- Primary outcomes: Changes in the expression of salivary biomarkers of bone remodeling
- Changes in rate of alignment of lower incisors

- Secondary outcomes:
  - Changes in tooth mobility
  - Changes in pain and Oral Health and Quality of Life

Chapter II: Hypotheses and Aims

A- Hypotheses and General Objectives

1- Hypotheses

1. The expression of specific biomarkers of bone remodeling in saliva is increased when fixed orthodontic appliances are combined with vibration.
2. The degree of tooth mobility is increased in patients with the combination of fixed orthodontic appliances and vibration.
3. The rate of incisor alignment is increased when fixed orthodontic appliances are combined with vibration.

4. Orthodontic patients using vibration devices daily experience less pain and improvement in the quality of life compare to those in the control group.

2- General objectives

There is a clear lack of evidence in the orthodontic literature about the effect of a vibration device on the speed to tooth movement. Additionally, the biological mechanism by which vibration may increase the rate of tooth movement is still unknown. The primary objective of this study is to assess the potential influences of vibration device on the expression of biomarkers of bone remodeling.

B- Specific Aims and Objectives

1- To determine if the addition of vibration to the regular fixed orthodontic appliances can alter the expression of biologic factors involved in bone remodeling.

2- To further elucidate the role of vibration treatment on the degree of tooth mobility during fixed appliance treatment compared to control group.

3- To determine if combined vibration-fixed appliance treatment increases the speed of orthodontic tooth movement during the alignment phase

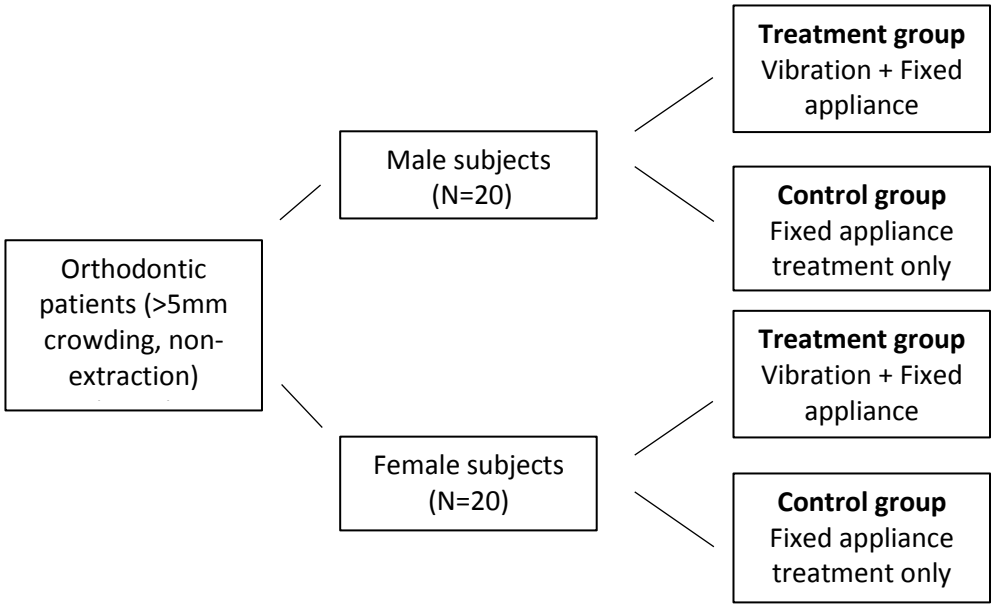
4- To evaluate the role of vibration treatment in the control of pain and quality of life in patients undergoing orthodontic treatment.

Chapter III: Materials and Methods

A- Study Design and Screening procedure:

1- Study design

This study was approved by the Institutional Review Board (IRB) of University of Connecticut (IRB #14-117-2). The aim of this study was to perform a randomized clinical trial recruiting a total of 40 patients equally and randomly divided in four groups: (1) 10 male subjects in control group; (2) 10 male subjects in vibration group; (3) 10 female subjects in control group; (4) 10 male subjects in vibration group.



No randomized clinical trials are currently available to predict vibration effects on the expression of biomarkers. Therefore, this study serves as a pilot research including 40 patients in 4 groups divided by gender and vibration/no-vibration treatment. This trial was registered in at Clinical Trials.gov (14-117-2).

2- <u>Screening & Recruitment Procedures</u>

Prospective subjects were screened for this study through the regular screening procedures followed by all new patients of the orthodontic clinic of University of Connecticut. The provider

assigned to the patient at the screening appointment determined if the patient was likely to qualify according to the inclusion/exclusion criteria and advised the study coordinator (MCC) if the clinical indicators were met. The initial eligibility requirements included any healthy male or female between 15-35 years old, not taking any medication, with good oral hygiene, with a minimum of 5 mm incisor crowding requiring a non-extraction treatment. If the prospective subjects met the initial criteria, the study coordinator then confirmed the possible eligibility by consulting the screening forms, models and/or radiographs. In a situation where the patient was between 15-17 years old, the initial provider asked the parent permission to provide the information to the study coordinator.

3- Enrollment

After the primary provider had determined the possible eligibility of a patient as well as verified his/her interest to participate in the trial, the study coordinator met with the potential subject on the next appointment (record appointment). The study was then explained to the subject in detail and informed consent was obtained by the patient himself and/or the parent (in the situation that the subject was under 18 years old). The patients had to meet the following inclusion/exclusion criteria in order to be enrolled in the study:

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	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
	Diseases 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

After enrollment, the patients were instructed not to use any anti-inflammatory medications during the course of the research and to not eat or drink for the duration of an hour prior to the appointment.

B- Study procedure

1- <u>Standardized Orthodontic Treatment Protocol</u>

The patients enrolled in the study had to follow a standardized protocol in order to minimize any possible variability that could affect the outcomes. All patients were bonded with passive self-ligating brackets (Carriere brackets) featuring 0.022"X0.025" slot and MBT prescription from second premolar to second premolar as well as a bonded tube on first molars. At the bonding appointment (T0), an 0.014" Cu-NiTi wire was inserted on the lower arch and was kept until the T2 appointment. At T2, bracket position was assessed by a

blinded provider and repositioning was performed as instructed by that provider. At this same appointment, the wire was changed for 0.014”X0.025” Cu-NiTi.

All subjects were seen for orthodontic adjustments every 5-6 weeks. If a bracket loosened, the patient had 7 days to advise his/her provider and the latter repositioned it to the ideal position. Failure to follow this protocol led to immediate disqualification from the study.

2- Randomization Procedure

Block randomization was chosen as the randomization technique for this study. Since study groups were subdivided by gender, separate randomization was performed for males and females. Twenty opaque envelops were included in each group (male and female) with 10 for control group and 10 for vibration group. During the bonding appointment (T0), the subject was asked to pick an envelope and disclose the allocated group. In the scenario of being assigned to the Acceledent group, instructions were given by the study coordinator regarding the operation of the device and they were told to use it 20 minutes per day for the whole study duration (3 months) according the to manufacturer’s instructions.

3- Data Collection Procedure

On the day of bonding, the baseline measurements were taken: unstimulated whole saliva was collected, Periotest was performed, alginate impression was taken and Oral Health Quality of Life questionnaire was answered. After fixed appliances were placed, the subjects were submitted to regular orthodontic treatment with or without the vibration device,

according to their group allocation. All measurements were taken once again at T1 (5-6 weeks), T2 (10-12 weeks) and T3 (15-18 weeks). Each subjects were seen at approximately the same time in the day in order to minimize the variables coming from the circadian rythms followed by salivary biomarkers.

- Salivary collection

Collection of unstimulated whole saliva was performed following the same protocol described by Navazesh and Kumar Quote. The saliva was collected into a sterile tube at baseline and then at each visit by passive drooling for 15 minutes or until 10 mL was reached. Proteinase inhibitor (Sigma-Aldrich, proteinase inhibitor cocktail, P2714) was then added to the accumulated saliva and centrifuged at 6000 rpm for 15 minutes to remove cellular debris and supernatants. This cocktail was made of AEBSF at 2 mM, Aprotinin at 0.3 µM, Bestatin at 116 µM, E-64 at 14 µM, Leupeptin at 1 µM and EDTA at 1 mM. At any time during the collection or the processing, the sample was kept on ice to assure preservation of the biomarkers. The samples were all stored in a -80ºC until biomarker analysis.

Biomarkers were assessed with the ELISA assay test using a direct sandwich method and standard protocol. A sample of primary antibodies with the desired selected factors was pre-coated on dishes. A secondary conjugated antibody was used to recognize binding with the use of chemiluminescence on the incubated sample product or standards. The targeted biomarkers include ALP, RANKL/TRANCE, OPG, Osteocalcin (bone formation marker), MMP8, MMP13, TNF α, IL1a, IL1b, IL3, IL6, IL11, and IL18. However, until now, only IL-1 β and IL-8 have been analyzed. Human antibody samples to these target

biomarkers were supplied by R&D Systems, inc. Furthermore, using a RatLaps kit (Immunodiagnosis System, Inc), salivary C-terminal telopeptide of type I collagen (CTX) (an indicator of bone resorption) will also be analyzed in the near future using the ELISA assay test.

- Cast Analysis

Dental casts were assessed by one blinded evaluator to determine the rate of tooth movement. Each mandibular model was evaluated for the mandibular anterior alignment from canine to canine, using Little's irregularity index. This index uses the displacement of the adjacent anatomic contact points of the mandibular incisors (mesial to right canine to mesial of left canine) in millimeters and determines the Irregularity Index of the subject by adding the five measurements together. [47] The measurements were measured on each model at T0, T1, T2 and T3 with a digital caliper held parallel to the occlusal plane and was evaluated over the 3 months' study period.

- Periotest Measurement

At each time points, the mobility of specific teeth of lower arch (central incisors, canines and second premolars) was assess with a device named Periotest (Siemens AG, Bensheim, Germany) as previously described by Liou et al [48]. The lower wire was removed and the tip of the device was held parallel to the floor, perpendicular to the tooth axis and 2 mm away from the labial surface. Each tooth was measured 3 times and the mean was recorded. The study coordinator located an area on the labial surface that had sufficient space for the tip to contact the teeth in order to take consistent measurements.

Teeth were out of occlusion during the recording. The values obtained by the Periotest can ranged from -8.0 to +50.0 and the unit of measure was "Periotest values". The scale correlates with Miller's index as shown in the table below: [49]

PTV Measure	Indication
-8.0 to +9.9	No movement distinguishable (Miller classification 0)
+10.0 to +19.9	First distinguishable sign of mobility (Miller classification I)
+20.0 to +29.9	Crown deviates within 1 mm of normal position (Miller classification II)
+30.0 to +50.0	Mobility easily noticeable (Miller classification III)

- Orthodontic Pain Assessment

Patients were instructed to fill a pain diary at the T0, T1 and T2 appointment to record the degree of pain experienced during their orthodontic treatment. It was assess using a Visual Analog Scale (VAS) ranging from 0 (no pain) and 10 (extreme pain) and was filled during the 7 days following their appointment. The completed diary was returned at the next appointment and stored by the study coordinator in the study record.

- Oral Health Quality of Life (OHQoL)

Patients were asked to complete an Oral Health Impact Profile (OHIP-14) questionnaire in order to measure subject's perceptions of the impact of oral conditions on their well-being as well as the possible impact of vibration device on it. This questionnaire included 14 questions that were divided into specific categories including functional limitation, physical pain, psychological discomfort, physical disability, social

disability and handicap. Each question was answered on a 5-point scale, ranging from 0 (never) to 4 (very often). The value was then multiplied by the weight attributed to it and added to the other questions of the same category to give a total score for each subgroup.

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

C- Statistics

Intrareliability of the irregularity measurements was assessed using the T0 and T3 models evaluated by the one blinded evaluator for all patients. The reliability of the measurement was then assessed by the use of Cronbach alpha analysis.

The Mann-Whitney Test was used to assess differences between groups for all the continuous variables with an $\alpha = 0.05$ for Periotest measurements, irregularity index changes, biomarkers concentration, VAS and OHIP-14 measures.

A non-parametric analysis was also performed using the Spearman's rank correlation coefficient to analyze possible association between salivary biomarkers expression and the change in the irregularity index.

Chapter IV: Results

Twenty-three patients were enrolled since the start of the project; of these, 11 (3 boys, 8 girls) were allocated to the Acceledent group and 12 (3 boys and 9 girls) were assigned to the control group. The enrollment started in June 2014 and is still in progress. Out of the 23 patients recruited, 3 patients of the control group were removed after enrollment: (see Figure 2): 1 female patient decided to continue her orthodontic treatment in another clinic, another failed to show at his third appointment and the last one had an emergency medical procedure which required the administration of anti-inflammatory drug, requiring exclusion from the study. The mean age of the participants allocated to the Acceledent and control group at the beginning of the trial was 20.6 and 21.0 years old, respectively. The initial irregularity means for the fixed appliances only was 9.1 (SD, 3.41) mm while the experimental group showed an average of 8.6 (SD, 3.92) mm, with no statistically significant different among the groups (P=0.817).

Table I shows the mean irregularity index at each time point for both groups. There was no statistically significant difference found between the experimental and control groups (P = 0.817, 0.763, 0.934, 0.544). In terms of the changes in irregularity over the 3 time points, the data which are represented in Table II did not show significant

differences for any of these periods (P = 0.900, 0.643, 0.716, 0.713, respectively). Multivariate linear regression was also performed to assess any potential correlations between the initial irregularity, age, sex and type of intervention on the reduction of the irregularity index. The only significant difference appreciated among the groups was attributed to the gender at T0 and T1, with the female group experiencing statistically significant less crowding. Also, when looking at the total alignment periods (T0-T3), there was a significant gender difference with females aligning less than males. In regards to patient compliance with Acceledent, based on the data recorded by the device, a great variability was observed in the percentage of use, varying from 2% to 102%, for a mean compliance rate of 63%. This result is in agreement with the 67% compliance reported by Kau et al. [15] The intra-reliability test showed excellent consistency with a Cronbach's Alpha value of 0.997 and 0.990 for T0 and T3 respectively.

The Visual Analog Scores (VAS) illustrated by the pain diary are represented in Table III. There was no significant difference in the level of pain intensity between both groups at T0, T1 and T2 (P = 0.775, 0.685, 0.100).

Table IV-V-VI show the tooth mobility changes collected with the Periotest device between each appointment. There was no statistically significant difference between the Acceledent and fixed appliances only groups at any time points. The highest increase in mobility was recorded between T0 and T1 and when comparing each tooth type, the highest changes were seen at the level of the incisors.

The evolution of OHQoL during orthodontic treatment was assessed using the Oral Health Impact Profile-14 (see Table VII). The initial results show steady means from T0 to T1 to finally reaching improved levels lower than the baseline values. There was no statistically significant difference between groups (P = 0.225, 0.565, 0.406, 0.565).

Up to now, temporal changes in the biomarker levels in the saliva were measured at each time points for IL-1β, IL-8 and TNF-alpha (see table VIII-IX). Since TNF-alpha concentration was found below the limits of detection in all samples, no statistical analysis could be performed. On the other hand, IL-8 and IL-1β were detected by the ELISA test and these two biomarkers showed no statistically significant difference between both groups at each time point. Furthermore, no correlation was found between the biomarkers and the changes in the irregularity index.

Chapter V: Discussion

Historically, comprehensive orthodontic treatment has been claimed to last approximately 21-27 months in non-extraction cases and 25-35 months when teeth are extracted. [1] Unfortunately, fixed appliance treatment, especially when duration is prolonged, can also result in harmful consequences such as white spot lesions [2], root resorption [3], gingival inflammation [4] and dental caries. To this date, research has focused on 3 main modalities to try and increase the rate of tooth movement and thus decrease the treatment time:

pharmacological, surgical and mechanical approaches. Although surgical modalities such as corticision, piezocision and Periodontally Accelerated Osteogenic Orthodontics have shown some positive data by taking advantage of the so called Regional Acceleratory Phenomenon, their invasive nature makes patients less inclined to consent to these treatment plans.

Some studies have investigated the effectiveness of the application of vibration during orthodontic treatment but up to now, no consensus has been made. In our research, no statistically significant difference between both groups was found either in the mean incisor irregularity at each appointment or in the changes in irregularity over the 3 time points. This result is in agreement with Miles et al [17] as well as Woodhouse et al [19] which both found in their respective study no increase in the rate of tooth movement when a vibration device was used. On the other hand, Pavlin et al [16] showed in a randomized clinical trial an average monthly rate of tooth movement of 1.16 mm/month when the AcceleDent appliance was used for 20 minutes daily, corresponding to an increase of 48% in the rate of space closure compared to their control group. However, it is primordial to be careful with the interpretation of their results since their design was slightly different, whereby they assessed the rate of space closure during canine retraction rather than the incisor alignment.

Pain is a common effect of orthodontic treatment and it is usually more significant immediately following appliance placement. Studies have shown that pain generally increases during the first 24 hours after adjustment appointment and then gradually reduces over a week [50] [51] [52]. In our study, analysis of the visual analogue scale confirmed this tendency in the level of pain felt by the patients at each time points, being the highest about

two days after the adjustment appointment and gradually reducing afterward. Other methods such as analgesic consumption record and questionnaires have also been reported in the literature to assess pain in orthodontic patients. [52]

Regarding the role of vibration device on pain level, previous studies have shown contradictory results. Lobre et al showed that the level of discomfort was significantly reduced by using this method. They mentioned that patients using the Acceledent device had lower scores for overall pain as well as biting pain during the 4 months' period of the study. [40]

Our findings are in disagreement with this previous study, showing no significant difference when patients were using a vibration device. Miles et al. showed similar results than us, with no significant difference between the groups in regards to pain at any of the time points during the study. [17] Furthermore, Woodhouse et al. in 2015 determined that the only significant predictor for mean pain was the time. Their data also showed that the use of Acceledent vibrational device did not have any significant effect on the pain level or analgesic consumption during the initial alignment phase. [19] Even though these two studies are consistent with our findings, more studies with higher sample sizes are needed to draw a definite conclusion on the subject. Interestingly enough, even though no significant difference in the pain experienced by the patient during orthodontic treatment was found in our study, three patients reported soreness on the teeth at the end of the daily 20 minutes of vibration, one of them having to stop using the device altogether due to severe pain.

Regarding the Oral Heath and Quality of Life, the overall scores stayed steady between the first and the second appointments, ultimately improving thereafter and reaching levels lower than the baseline values. Similar to the pain level, we did not find any significant differences between the two groups. These findings are in agreement with previous research which showed that some patients go through a transitional phase of deterioration of the OHQoL during the active orthodontic phase [44] [45]. This result might be explained by the fact that following the bonding appointment, the patient can be self-conscious about the appearance of the fixed appliances, which would increase the overall score of the Oral Health Impact Profile (OHIP-14) questionnaire. Furthermore, the lack of a statistically significant difference found might also be related to the small sample size. Conversely, a study performed by Collado-Mateo et al. showed that whole body vibration could be an adequate treatment for fibromyalgia, improving balance, disability index and health related to quality of life as well as positively affecting fatigue and pain. [53] To our knowledge, this study is the only one that compares the quality of life with the usage of vibration during orthodontic treatment. To assess psychosocial impacts as well as the possible factors that could contribute to improve the overall experience of the patients during treatment, further studies with larger sample sizes are needed.

It is well known that the orthodontic tooth movement is a metabolic event featuring a combination of bone resorption on the compression side and bone apposition on the tension side. This alteration in the alveolar bone turnover is usually clinically associated with increased tooth mobility. In 2011, Liou et al. published an article in which they assessed the

postoperative changes in bone metabolism after orthognathic surgery and the corresponding responses in the dentoalveolus, such as the changes in tooth mobility. [48] In their 4 months' postoperative evaluation, one of the main findings included an increase tooth mobility between the first week and third month follow-up appointment, coinciding with the results appreciated in our research. Indeed, the mobility values obtained during our three-month trial showed an overall increase in mobility when an orthodontic force was applied, the highest increase in mobility being appreciated between T0 and T1, at the level of the incisors. However, no statistically significant difference was found between the fixed appliances only group and the group applying vibrational force daily. To our knowledge, this is the first study assessing the amount of mobility change during tooth movement. Consequently, considering the small sample size, it would be of rudimental importance to perform more research on the subject including a much bigger sample size before drawing any conclusions.

Proinflammatory cytokines have successfully demonstrated to comprise an important role during remodeling of the alveolar bone by regulating the inflammatory process during orthodontic tooth movement. There has been a recent increase in research interest in this field to try to fully elucidate the process of tooth movement. Rats [25] and humans [26] research focusing on Tumor Necrosis Factor alpha (TNF-α) and interleukins concentration have shown increased values when orthodontic force was applied. In our research, TNF-α concentration was found to be below the limits of detection in all samples. The discrepancy in this finding could be however explained by the fact that different methods of collection were used, Basaran et al. using gingival crevicular fluid instead of saliva. This difference in

the protocol could affect some biomarker detection, especially ones found to be expressed in lower concentrations in the GCF.

In a study performed in 2007, Ren et al. measured a panel of proinflammatory cytokines (IL-1β, IL-6, IL-8 and TNF-α) during tooth movement of short and long durations and found large variation in the results for each biomarker [27]. They found statistically significant increased levels of IL-1β, IL-6 and TNF-α in the GCF 24h post-force application however these values were slowly subsiding and returning to baseline levels by the end for the month. On the other hand, they found a different trend in the concentration of IL-8 in the long-term, where it reached a significant elevation in GCF after 1 month of tooth alignment, eventually decreasing back to the baseline values at 2 months. Similar results were found by Lee et al. in a study performed on Wistar rats where they concluded that the application of an orthodontic force lead to a significant increase in IL-1β in pressure side gingiva on day 7 and 14 [28]. Regarding our findings, because of the small sample size, it is difficult to identify and confirm possible trends. While the IL-1 β showed wide variability in the concentrations, the IL-8 results tend to decrease expression after 1 month, later increasing at the 3rd month time point. Comparing both groups together, we also found no statistically significant differences in regard to proinflammatory cytokine levels when a vibration device was used compared to the fixed appliance only group. It however needs to be kept in mind that factors such as the circadian rhythm and the presence of oral inflammation have been shown to have an effect on cytokine expression. [54] To our knowledge, this study is the first one to evaluate the proinflammatory cytokines while applying vibration to tooth movement, which

demonstrate the need of more research in this field. Furthermore, due to the small sample size, it is too early draw any definitive conclusions on the subject.

Chapter VI: Conclusion

1. There was no statistically significant difference in the expression of biological markers of bone remodeling between the Acceledent and the control group.

2. There was no difference in the degree of tooth mobility in patients undergoing combined vibration-fixed appliance treatment compared to orthodontic treatment alone.

3. The application of vibration to the dentition during orthodontic treatment did not show greater changes in the irregularity index at any time point during the study.

4. The difference in the level of pain and Oral Health and Quality of Life was not statistically significant in patients undergoing combined-treatment with a vibration appliance compared to controls.

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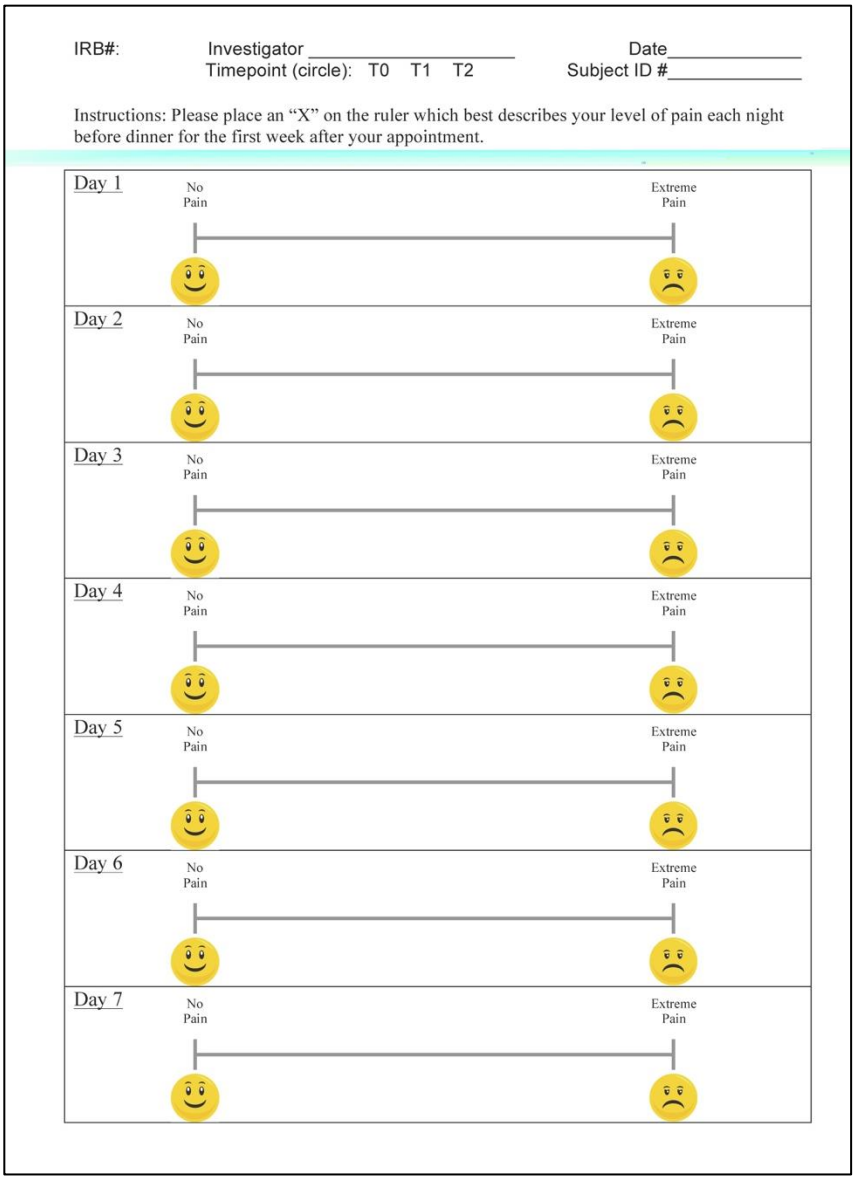
FIGURES

Figure 1. Pain Diary



IRB#: Investigator _____ Date_____
Timepoint (circle): T0 T1 T2 Subject ID #_____

Instructions: Please place an "X" on the ruler which best describes your level of pain each night before dinner for the first week after your appointment.

Figure 2. Consort flow diagram for patient participation

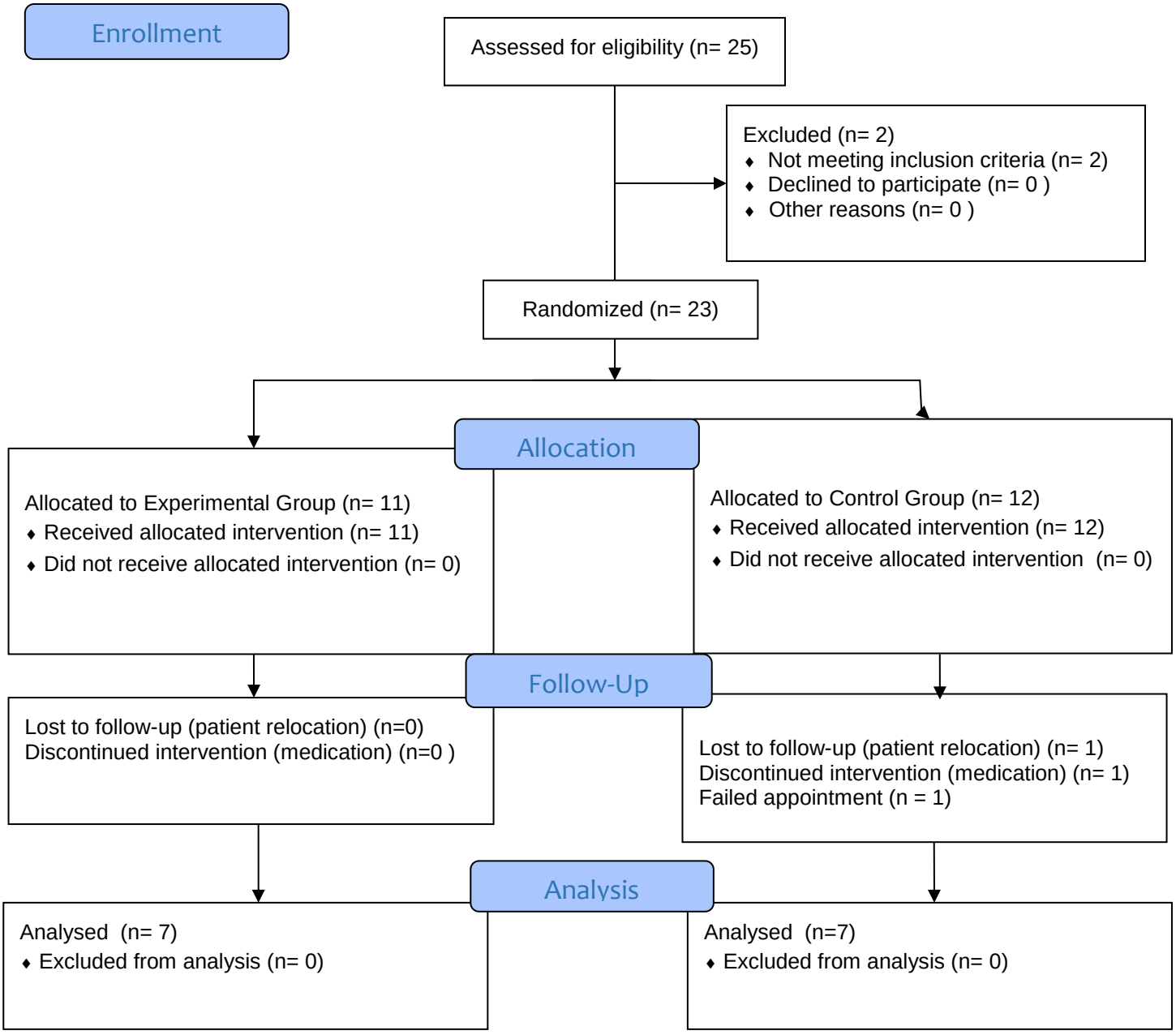
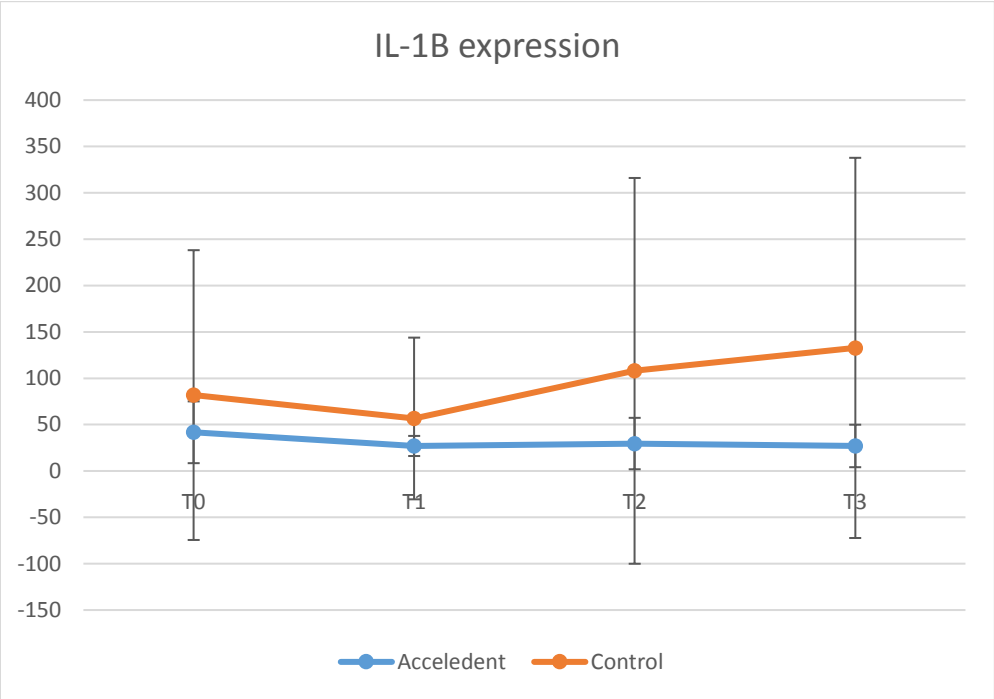
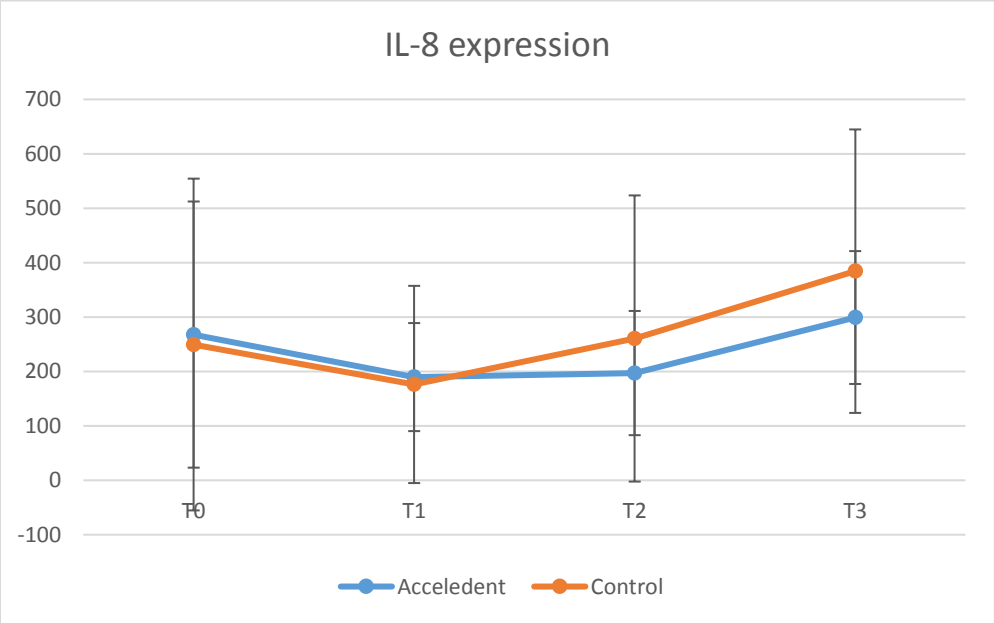


Figure 3. Salivary IL-1B expression



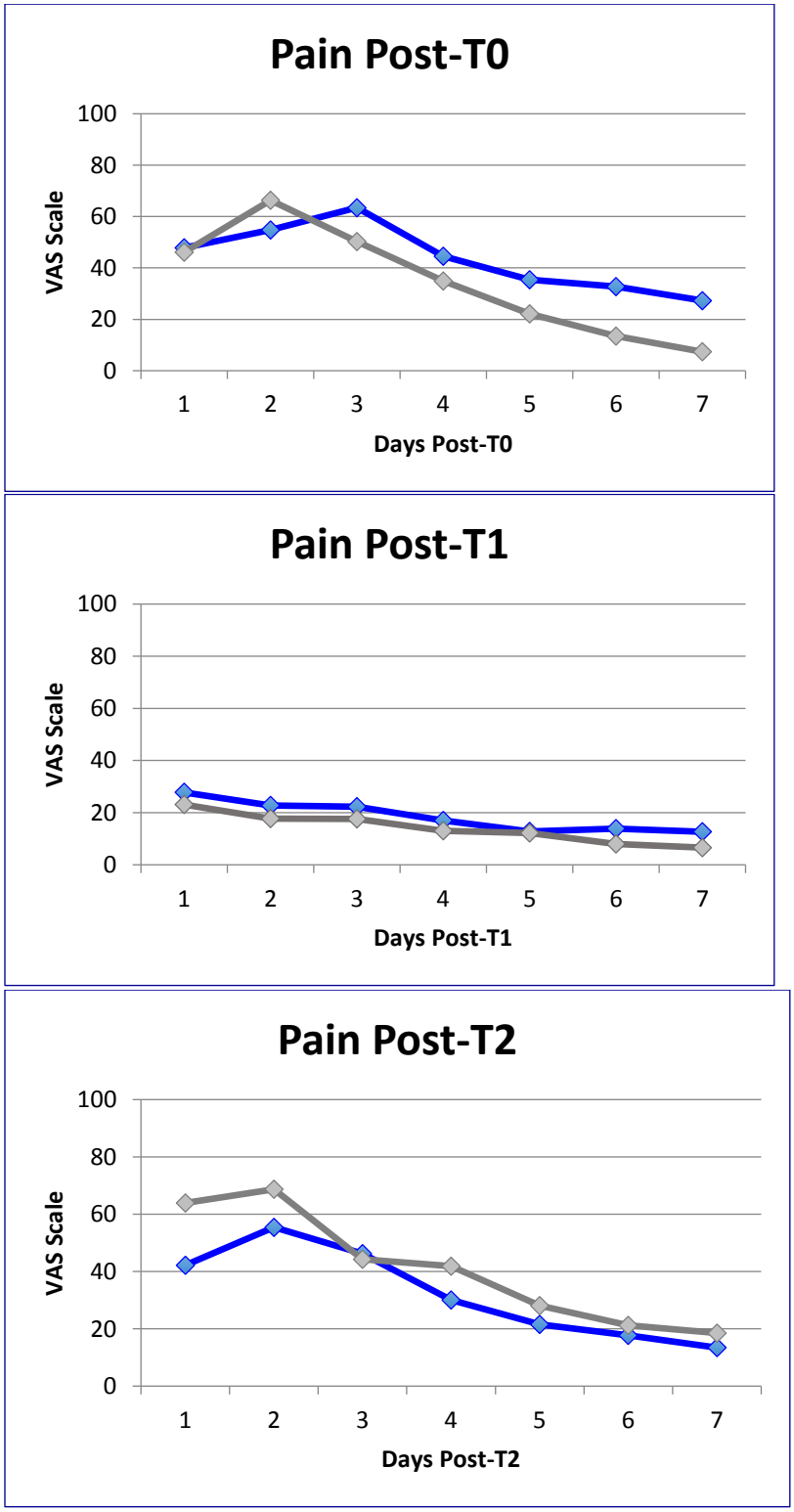
pg/mL

Figure 4. Salivary IL-8 expression



pg/mL

Figure 5. Pain Dairy Score



TABLES

Table I. Irregularity index at each time points

	Acceledent		Control		P value
	Mean	SD	Mean	SD	
T0	8.61	3.92	9.10	3.41	0.817
T1	5.29	4.02	5.88	2.54	0.763
T2	2.96	1.98	2.88	1.15	0.934
T3	1.23	1.41	0.85	0.72	0.544

Table II. Irregularity changes between each time points

	Acceledent		Control		P value
	Mean	SD	Mean	SD	
T0-T1	3.32	1.29	3.22	1.59	0.900
T1-T2	2.33	2.61	2.99	2.40	0.643
T2-T3	1.73	1.84	2.04	0.84	0.716
T0-T3	7.38	4.43	8.25	3.79	0.713

Table III. Pain scores in the experimental and control groups (%)

	Acceledent		Control		P value
	Mean	SD	Mean	SD	
T0	40.94	28.18	34.37	14.16	0.775
T1	16.92	22.20	14.00	16.33	0.685
T2	28.28	24.62	40.96	22.25	0.100

Table IV. Periotest values changes between each time points (incisors)

	Acceledent		Control		P value
	Mean	SD	Mean	SD	
T1-T0	7.84	6.72	2.84	4.67	0.132
T2-T1	-4.11	3.37	-0.29	4.91	0.115
T3-T2	-0.16	4.19	1.92	2.50	0.281
T3-T0	3.56	4.41	4.48	3.65	0.681

Table V. Periotest values changes between each time points (canines)

	Acceledent		Control		P value
	Mean	SD	Mean	SD	
T1-T0	2.60	1.04	2.67	1.66	0.931
T2-T1	-0.49	1.53	-0.64	1.41	0.859
T3-T2	1.52	1.61	0.94	2.01	0.566
T3-T0	3.62	2.58	2.97	1.75	0.591

Table VI. Periotest values changes between each time points (premolars)

	Acceledent		Control		P value
	Mean	SD	Mean	SD	
T1-T0	1.28	1.37	0.35	1.51	0.254
T2-T1	-0.28	2.81	0.83	1.47	0.373
T3-T2	0.08	1.37	0.24	1.81	0.863
T3-T0	1.09	2.47	1.42	1.73	0.775

Table VII. Oral Health Quality of Life scores

	Acceledent		Control		P value
	Mean	SD	Mean	SD	
T0	5.24	4.96	7.33	4.06	0.225
T1	5.50	3.27	7.27	3.26	0.565
T2	4.40	4.19	5.52	3.30	0.406
T3	3.18	2.79	4.71	2.88	0.565

Table VIII. IL-1B salivary expression

	Acceledent		Control		P value
	Mean	SD	Mean	SD	
T0	41.72	33.32	81.85	156.26	0.406
T1	26.97	10.84	56.62	87.21	0.749
T2	29.57	27.75	107.98	208.04	0.749
T3	26.98	22.92	132.73	205.01	0.749

Table IX. IL-8 salivary expression

	Acceledent		Control		P value
	Mean	SD	Mean	SD	
T0	267.80	244.60	249.49	304.87	0.848
T1	189.65	99.29	176.12	181.22	0.406
T2	197.06	114.16	260.59	262.95	0.949
T3	299.11	122.12	384.32	260.50	0.655