

6-27-2012

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Hamed Vaziri

HaVaziri@gde.uchc.edu

Recommended Citation

Vaziri, Hamed, "The Effect of Corticision on The Rate of Orthodontic Tooth Movement in Rats" (2012). *Master's Theses*. 349.
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**The Effect of Corticision on The Rate of Orthodontic Tooth
Movement in Rats**

Hamed Vaziri

D.M.D., University of Pennsylvania, 2009

A Thesis Submitted in Partial Fulfillment of the Requirements

For the Degree of

Master of Dental Science

at the

University of Connecticut

2012

APPROVAL PAGE

Master of Dental Science

The Effect of Corticision on The Rate of Orthodontic Tooth Movement in Rats

Presented by

Hamed Vaziri, D.M.D.

Major Advisor

Flavio Uribe, D.D.S., M.D.S.

Associate Advisor

Ravindra Nanda, B.D.S, M.D.S., Ph.D.

Associate Advisor

Ivo Kalajzic, M.D.Ph.D.

University of Connecticut

2012

TABLE OF CONTENTS	Page
Title page	1
Approval page	2
Table of content	3
List of figures	4
Abstract	5
I) Introduction	7
A. Background	7
1. Anatomy, Biological Responses, and Orthodontic Tooth Movement	7
2. Tooth Movement Models	11
B. Rationale and Objectives	16
II) Hypotheses and Aims	16
A. Hypotheses and General Objectives	16
B. Specific Aims/Objectives	17
III) Materials and Methods	17
Rat Tooth Movement Model	18
Method for orthodontic force application	18
Tooth movement measurement	20
Application of corticision	20
Wellness monitoring and Euthanasia	20
Micro-CT Analysis	21
Dissection and tissue preparation	21

	Histological analysis	22
	Statistics	23
IV)	Results	23
V)	Discussion	26
VI)	Summary and Conclusion	31
VII)	Figures	32
VIII)	References	42

List of Figures

- Fig 1.** Placement of the closed coil spring in an *In vivo* rat tooth movement model.
- Fig 2.** Localization of corticision in relation to maxillary first molar.
- Fig 3.** Micro-CT image of the dissected maxilla following OTM.
- Fig 4.** Evaluation of intermolar distance following OTM.
- Fig 5.** Histological quantification of active osteoclasts on the alveolar bone.
- Fig 6.** Graph representing the osteoclast surface parameter.
- Fig 7.** Histological quantification of active odontoclasts.
- Fig 8.** Detection of RANKL expression by Immunohistochemistry.

ABSTRACT

Minimal treatment time is a primary goal for orthodontic therapy. Reducing treatment time decreases risk of caries, periodontal disease, root resorption, and increases patient satisfaction. Orthodontic tooth movement (OTM) is a result of bone resorption and apposition; factors influencing the rate at which these processes take place may affect OTM. Various attempts have been made to shorten treatment time. The regional acceleratory phenomenon (RAP) has been used to modify the balance between resorption and apposition of bone through selectively injuring the cortical plate of the alveolus to accelerate tooth movement. This approach is limited due to the need of flap reflection affecting the acceptance of this treatment modality by patients due to the invasive nature of the procedure. In order to induce RAP without flap reflection a new approach called corticision has been introduced, which consists of transmucosal manipulation of the alveolar bone with a scalpel incision without flap reflection.

Since any manipulation of alveolar bone can potentially increase RAP and hence accelerate bone remodeling, we hypothesize that corticision will increase the rate of orthodontic tooth movement. In order to test this hypothesis we will use an *in vivo* rat orthodontic tooth movement model. Our specific aims are:

Specific Aim 1: To determine if corticision enhances the rate of orthodontic tooth movement.

Our working hypothesis for this aim is that the rate of tooth movement will be increased by performing corticision.

Specific Aim 2: To determine the number and localization of the osteoclasts and evaluate the expression of receptor activator for nuclear factor K B Ligand (RANKL) during orthodontic tooth movement with corticision.

Our working hypothesis for this aim is that corticision will increase osteoclastogenesis. In order to test our working hypothesis, we will localize and evaluate the osteoclast numbers and expression of RANKL by immunohistochemistry.

Chapter I: Introduction

A. Background

1. Anatomy, Biological Responses, and Orthodontic Tooth Movement

Orthodontic treatment usually requires approximately two years of intervention to full completion. An increased risk of problems due to caries, periodontal disease and root resorption are associated with prolonged treatment times. Reducing orthodontic treatment time is one of the primary goals for orthodontists as it leads to increased patient satisfaction. Since orthodontic tooth movement (OTM) is caused by a gradual remodeling (apposition and resorption cycle) of supporting alveolar bone, factors affecting this cycle could modulate the rate of tooth movement [1].

The attempt to shorten the treatment time can be divided into different categories. Local or systemic administration of biologic factors [2, 3] such as parathyroid hormone (PTH) [4], thyroxine [5], Vitamin D₃, [1,25 (OH)₂D₃] [6] and prostaglandins [7]. The pharmacological approaches that have been shown to increase tooth movement have also resulted in numerous adverse reactions, such as, local pain [8], severe root resorption [9], and drug-induced side effects. For this reason, the trend has turned towards finding a physical or mechanical approach that can accelerate tooth movement without side effects. These approaches include, but are not limited to: electrical currents [10, 11] magnets[12], laser beams [13] and vibration[14]. The treatment designs which have recently received most attention involve the surgical manipulation of bone

using either dental distraction [15], alveolar surgery to undermine interseptal bone [16], corticotomies [17], osteotomies [18] and the most recent approach, corticision [19, 20].

All these approaches are focused on controlling the microenvironment of alveolar bone in an attempt to reduce tissue resistance. This intentional osteopenia is a method of physical, rather than pharmacological, induction of tooth movement. In 1892 this method involving corticotomies of the alveolar bone was first introduced, but was not clinically applied until 67 years later by Kile [21-23]. In 1959 he surgically operated on the alveolar process and removed a portion of the cortical bone to correct malocclusions. Then in 1991 Suda [24] further refined this method and termed it, "Corticotomy-Facilitated Orthodontics" (CFO). In 2001 Wilcko and Wilcko [25] popularized this method as "Wilckodontics." Notably, this rapid acceleratory phenomenon is also witnessed in fracture healing [26, 27]; a redirection of this normal physiologic bone response to insult has been exploited to mobilize and accelerate tooth movement [25].

The biological mechanism of these different stimuli is based on the regional acceleratory phenomenon (RAP) first described by Frost in 1983 [28]. The characteristics of this localized remodeling process have accelerated healing particularly following the surgical wounding of cortical bone which also accelerates bone turnover and modeling [28]. RAP starts in alveolar bone with an initial burst of osteoclast activity which decreases bone density followed by enhanced osteoblastic activity which increases bone density [29]. It has been

shown that osteoclast activity is important in tooth movement. Factors that can decrease this activity like bisphosphonates can decrease the rate of tooth movement [30]. On the other hand, factors that can increase this activity and decrease bone density can be expected to result in faster tooth movement. In a comprehensive study Baloul et al.[31] showed that selective alveolar decortication increases osteoclastogenesis as evidenced by the increased expression of RNA markers of osteoclastic cells and osteoclastic key regulators such as RANKL, M-CSF (macrophage colony stimulating factor), osteoprotegerin, CTR (calcitonin receptor), TRACP-5b (tartrate-resistant acid phosphatase 5b) and cathepsin K. In another, Teixeira et al.[32] examined 92 cytokines during orthodontic tooth movement and showed that 37 of them were increased significantly during OTM. They also showed that adding small perforations in the cortical bone increases most of those cytokines even to the higher level.

In a recent study on foxhounds by Sanjideh et al. [33], alveolar corticotomy significantly increased orthodontic tooth movement, and at the peak of velocity the rate of tooth movement was 85 per cent faster compared to the control side. It was observed that this effect was transient; it had a peak followed by a decrease. This was most probably due to a transition from the catabolic to the anabolic phase of RAP when density of bone is minimum and the resistance to tooth movement is least [33]. In the same study, performing a second corticotomy helped to maintain higher rates of tooth movement for a longer period. However, the authors concluded that the cost benefit of a second

corticotomy procedure was not justified since flap reflection can cause crestal bone resorption and bone dehiscence. Moreover, patient acceptance can be challenging due to the invasive nature of the procedure [33].

In an attempt to find a less invasive procedure, Kim et al. [19, 20] introduced a technique called corticision which is a transmucosal manipulation of the alveolar bone with a reinforced scalpel without flap reflection. This study demonstrated that performing corticision in a feline model caused extensive direct resorption of bundle bone with faster removal of hyalinized tissue, which is the initial obstacle to orthodontic tooth movement, compared to the control group. It also showed that corticision accelerates the anabolic remodeling. Histological analysis showed neither pathologic changes nor root resorption following this technique.

Compared to the drug- induced approach, a major benefit of surgical assisted orthodontics is that the main effects of RAP seem to be restricted to the site of stimuli and even areas of close proximity seem to be unaffected [33-36].

Using these surgical techniques to accelerate tooth movement creates the potential for utilizing a model of differential anchorage. Certain teeth could be targeted with surgical incisions for rapid tooth movement while other teeth at other sites left uncut would be preserved for orthodontic anchorage.

The purpose of this study is to assess the biologic effects of corticision on remodeling of alveolar bone in orthodontic tooth movement in a rat model.

2. Tooth Movement Models

Historically, several animal models have been designed to study tissue responses to mechanical loading during orthodontic tooth movement. Primate, dog and cat models have been reported in pioneering histological studies using light microscopy [37, 38] and electron microscopy [37, 39]. The limitations related to the use of these animal models are directly due to their similarity and applicative value to humans. The rat model proposed by Waldo in 1954 [40] had increased levels of experimental control over other animal models and has become the investigative workhorse for unraveling the processes of mechanotransduction and alveolar bone remodeling in orthodontic tooth movement [41]. Today, rats are the most commonly used animal model, accounting for over half of all orthodontic tooth movement animal studies [41]. Compared with most other animals, the use of the rat has several advantages: they are relatively inexpensive, which allows using large samples; they can be housed for long periods of time, histological preparation of the rat is easier than other models; there is greater availability of antibodies required for cellular and molecular biological techniques and they are larger than mice, which makes it easier to place orthodontic appliances. The rat does have its own limitations: denser alveolar bone as compared to humans, the lack of osteons and less abundant osteoid tissue, structural dissimilarities in the arrangement of PDL fibers and the supporting structures while tissue development during root formation and tissue changes incident to orthodontic treatment appear to be

faster in rats than in humans, although their principal mechanisms are the same [41].

Rat models have enabled a diverse scope of orthodontic research, ranging from measuring proliferation rates of periodontal cells under load to assessing the effects of prostaglandins, bisphosphonates and leukotrienes on tooth movement [7, 30, 42].

In Ren et al.'s systematic review of the 153 (57% of the total tooth movement models) studies done on rats over the past twenty years it was found that the majority of the experimental models utilized poorly designed force systems that lacked control over force levels and constancy over the duration of tooth movement [41].

Only three methods met Ren's inclusion criteria for a good model [41]. Ren's inclusion criteria were: a force magnitude of less than 20cN; mesial movement of molars; an experimental duration greater than 2 weeks; and no extra experimental conditions, such as drug intervention. Most of the studies failed to take into account the physiology of the rat (i.e. natural distal drift of the molars and the continual eruption of the incisors), or the orthodontic appliance design was faulty. The distal drift of the molars underestimates the amount of mesial movement of the molars; continual eruption of the incisors can lead to a minimized control of force direction. The appliance design can be considered poor when it does not take into account the 50 fold decreased rat molar root surface area compared to humans, or it lacks a constant and continual force [41].

Pavlin et al. performed experiments to test the load conditions that would generate an optimal biological response of paradental tissues [43, 44]. They used an elastomeric “o-ring” tied between maxillary incisors and the first molar, and a red elgiloy (alloy of nickel and cobalt) open coil spring (0.0056 x 0.022 inches, Rocky Mountain Orthodontics, Denver, CO) tied and bonded to the same teeth, respectively. It was found that the coil spring has considerable advantages over the “o-ring.” Firstly, bonding of a coil spring to the molar and the incisors eliminates contact of the appliance with gingival tissues, greatly reducing the risk of tissue irritation [43, 44]. This correlates with the criticisms of Charles Waldo, whom in 1954, was among the first pioneers responsible for the advent of the rat model. His method, known as the Waldo method, utilized an orthodontic intermaxillary elastic, which was stretched and inserted into the interproximal space just cervical to the contact area between the molars of rats [40]. This method has been criticized due to the unknown force decay of the elastic. Springs have proven to be more reliable, able to deliver a reproducible force of 10 +/-2CN over a range of 3-15mm of activation [41]. Secondly, the spring has a lower force/deflection rate (F/Δ). This allows for a more precise and reproducible application of a low level force, which also remains more constant compared with that delivered by an elastomeric “o-ring.”

King [45], Keeling [46], and Nixon [47] in the 1990's produced the only 3 articles that met all of Ren's criteria for an ideal rat model [41]. Forces of 20, 40, and 60cN were used in all 3 articles. These studies were criticized for having an initial constant force, but not reactivating it, and forces of 40 and 60cN being too

high. The appliance consisted of a 9 mm length of NiTi closed coil spring (0.006 x 0.022 inches, Unitek, Monrovia, Calif.) suspended between a cleat bonded to the occlusal surface of the maxillary first molars and the lateral surface of the maxillary incisors. Initial force values were measured by suspending known weights from the anterior end of these coils before fixation to the incisors. Tooth movement was based on enlarged cephalograms, and was measured from the position of a reproducible landmark on the molar cleat with respect to either zygomatic amalgam implants, or a barbed broach placed submucosally on the palate. Palatally placed barbed broaches represented a more reliable, less traumatic, and more easily executed superpositional landmark than zygomatic amalgams. They only had a 79% appliance success rate, the animals lost weight, and they extracted mandibular first and second molars. All of these factors contributed to poor overall animal care [41, 45-47].

In 2004, Ren's model was fabricated due to the shortcomings of the rat models used from 1981-2002, and used a spilt-mouth design. This design compensated for the physiological distal drift of the molars, growth of the snout and concomitant forward movement of the incisors, and the continuous eruption and possible distal tipping of the incisors. Stainless steel ligature wires with a diameter of 0.2 mm were bent to enclose all three maxillary molars as one unit. To this ligature wire a Sentalloy® closed coil spring (Ni Ti, 10 cN, wire diameter 0.22 mm, eyelet diameter 0.56 mm, GAC, New York, USA) was attached to deliver a reproducible force of 10 ± 2 cN over a range of 3-15 mm activation. A transverse hole was drilled through the alveolar bone and both maxillary incisors

at the mid-root level using a drilling bur (D0205, Dentsply, Montigny le Bretonneux, France). A stainless steel ligature wire (diameter 0.3 mm, Dentaureum, Pforzheim, Germany) was inserted through the hole. Bonding was applied until the buccal and palatal wires were entirely embedded in the bonding material, then it was light cured. It was activated and subsequently attached to the ligature wire through the snout and the incisors [41].

Most recently, in 2006, Yoshimatsu et al. used a variation of the Ren model using NiTi closed coil springs [48]. Their mouse model included a NiTi closed coil spring, with the wire diameter of 0.15mm, and the coil diameter 0.9mm. The appliance was inserted between the maxillary incisor and the first molar on the left side. It was fixed with a 0.1mm wire around each tooth using a dental adhesive agent (Superbond; Sunmedical Shiga, Japan). To prevent detachment from the maxillary incisors during the experiment, a shallow groove, 0.5mm from the gingiva, was made on the maxillary incisor every 4 days, and the wire was reattached at the new groove. According to the manufacturer's database, the force level of the coil spring after activation was approximately 10g. The maxillary left molar was used as the experimental side, and the right as the control, taking into account the distal molar drift that would naturally occur [48].

B. Rationale and Objectives

Surgical injury is a potentiating factor for the induction of regional acceleratory phenomenon and the use of these supplemental dentoalveolar surgeries to accelerate orthodontic tooth movement has been recommended. In 2009 Kim et al.[20] introduced a technique called corticision shown to increase the rate of orthodontic tooth movement in a feline model. Therefore the goal of this study is to determine the effect of corticision on orthodontic tooth movement and to quantify and localize the osteoclasts and osteoclastogenesis induction molecules (RANKL) responsible for the biological response.

Chapter II: Hypotheses and Aims

A. Hypotheses and General Objectives

Hypothesis 1: We hypothesize that there will be greater orthodontic tooth movement in the corticision experimental group, than the control group.

Hypothesis 2: We hypothesize that there will be more osteoclasts and higher RANKL expression in the corticision group than the control group.

Null Hypothesis 1: There will be no difference in the amount of tooth movement in the corticision experimental group versus the control group.

Null Hypothesis 2: There will be no difference in quantity of osteoclasts or RANKL in the corticision experimental group versus the control group.

B. Specific Aims/Objectives

Aim 1: To determine the effect of corticision and number of corticisions on the rate of orthodontic tooth movement.

Aim 2: To determine the quantification and localization of the osteoclasts and RANKL during orthodontic tooth movement after corticision.

Chapter III: Materials and Methods

All experiments were performed under an institutionally approved protocol for the use of animals in research (University of Connecticut Health Center #2007-341). This was an experimental study with 32 rats in total. These rats were randomly placed into four groups (8 in each group). All groups received an orthodontic appliance. These groups included: (1) control: no corticision; (2) 1C: corticision at the time of appliance insertion; (3) 2C: one corticision at the time of appliance insertion and another one a week after; (4) gingival: no corticision but gingival cut at the place of corticision at the time of appliance insertion.

Rats were subjected to the application of orthodontic force from the maxillary left first molar to the central incisors. The rats were weighed every week in order to ensure that they were eating normally. Any rat that lost more than 20% of their weight in one week, or had weight loss in two consecutive weeks, was sacrificed and excluded from the study.

Upon completion of the research study, the rats were euthanized by CO₂, followed by cervical dislocation. All animal experimental procedures were in compliance with the guidelines in the care and use of animals in the American Journal of Physiology and the University of Connecticut Health Center.

Rat Tooth Movement Model

Young male, Sprague Dawley rats (Charles River 6 weeks, body weight 150-250g) were used for the experiments. The animals were housed under normal laboratory conditions, and fed with the standard powdered food (equal to standard rat chow) provided by center for comparative medicine and water ad libitum. The food was checked and changed every day. A standard 12 hour light and dark cycle was maintained. The animals were acclimatized for at least 1 week before the experiment start.

Method for orthodontic force application

Animals were placed under general anesthesia with xylazine (13mg/kg) and ketamine (87 mg/kg). A low force/deflection rate nickel titanium coil spring delivering 60 g of force was used for the application of orthodontic force. The force/deflection rate (F/Δ) for the spring was determined in order to calibrate the amount of force produced by activation of the spring.

Prior to appliance delivery a 0.014 mm SS ligature was threaded through the contact between the first and second left maxillary molars. Self-etching primer (Transbond Plus self etching primer, 3M Unitek) was applied to the lingual surface of the first molar, and a ligature bonded with light-cured dental adhesive resin cement (Transbond 3M Unitek), with a commercial unit (LEDemetron 1, Dentsply). The spring was then attached to the 0.014 mm SS ligature around the first molar and activated to the incisors. A second 0.014 mm SS ligature was placed around the incisors, spring was activated, and reinforced with the same bonding procedure as the molar. In addition, grooves 0.5mm from the gingival margin were prepared on the facial, lingual, and distal surfaces of the maxillary central incisors to prevent the ligatures from dislodging from the incisor due to their lingual curvature and eruption pattern. After the ligatures being tied and cut, composite resin (Transbond XT Light Cure Adhesive Paste, 3M Unitek, Monrovia, CA) was placed over the wire to prevent slipping and gingival irritation, as well as pulpal irritation due to exposed dentin. In order to minimize the distal movement of the left incisor and reinforce the anterior anchorage, the right and left incisors were joined to act as a unit. Finally, the mandibular incisors were reduced to prevent appliance breakage [49] (Fig 1). After appliance insertion the rats were allowed to recover in the presence of an incandescent light for warmth and the animals were returned to their cages once full ambulation and self-cleansing returned. The appliance was checked twice weekly, and additional bonding material was added if necessary.

Tooth Movement Measurement

After the animals had been sacrificed and before the springs were removed tooth movement was determined by measuring the space created between the first and second molars with a standard millimeter feeler gauge (Mitutoyo, Japan).

Application of corticision

After induction of anesthesia, corticision was performed on the mesio-palatal aspect of the maxillary left first molar in the corticision groups. A tip of the reinforced surgical blade (No. 11, Bard-Parker, NJ, USA) capable of making a surgical incision with a minimum thickness of 400 μm was employed. The blade was positioned on the gingiva 0.5mm from the corresponding tooth surface at an inclination of 45°–60° to the long axis of the maxillary first molar. The blade was inserted gradually into the bone marrow penetrating the overlying gingiva, cortical bone, and cancellous bone (Fig 2).

Wellness monitoring and Euthanasia

Rats were subjected to the application of orthodontic force from their molars to their central incisors. In addition some of the rats received corticision. In order to ensure that the rats were eating customarily, they were weighed every

week. Any rat that had lost more than 20% of their weight in one week or that had weight loss for two consecutive weeks was sacrificed and excluded from the study.

Upon completion of the research study, the rats were euthanized by CO₂ followed by cervical dislocation.

Micro-CT Analysis

Micro-CT analysis was performed by the micro-CT facility at the University of Connecticut Health Center for one rat per group. Scans were performed at 55 kV and 145 mA, collecting 1,000 projections per rotation at 300 millisecond integration time. Three-dimensional images were constructed using standard convolution and back projection algorithms with Shepp and Logan filtering and rendered within a 12.3 mm field of view at a discrete density of 578,704 voxels/mm³ (isometric 12 mm voxels) (Fig 3).

Dissection and Tissue Preparation

After decapitation, the mandibles were removed. The maxilla was then hemisected, and cleansed of soft tissues and muscles. The hemisected maxilla subsequently was placed in 10% Formalin for five days at 4°C with constant agitation. Then the samples were washed with tap water for 5 min, decalcified in 14% EDTA for 4 weeks and then processed for standard paraffin embedding. Serial sagittal sections 5 µm thick of the maxilla were cut and stained with hematoxylin and eosin (H&E).

Histological analysis

Immunohistological analyses were performed on all experimental groups. Tissue sections were deparaffinized with xylene and rehydrated with decreasing concentrations of ethanol. Following rehydration, the sections were treated with 0.3% peroxide for 15 minutes to block endogenous peroxidase activity. In order to unmask the specific antigen, tissues were incubated in 1 x citrate buffer (pH 6.0) at 60°C overnight. The following day, tissues were washed in PBS and blocked with 10% donkey serum in 1% bovine serum albumin for 2 hours. Incubation with the primary antibody was performed at 4°C overnight using goat polyclonal anti RANKL antibody (sc7628) at a concentration of 1:100. The next day, tissues were washed in PBS and incubated with a donkey secondary antibody (sc 2020) at a concentration of 1:150 for 1 hour. Staining was developed using peroxidase substrate (3,3'-diaminobenzidine).

Paraffin sections were stained for tartarate-resistant acid phosphatase (TRAP) activity using an acid phosphate leukocyte kit (Sigma Chemical, St Louis, MO) according to the manufacturer's instructions. Osteoclasts were considered as TRAP-positive multinucleated cells (2+ nuclei) and were counted on the alveolar bone surface of the compression side of the disto-buccal root. Histomorphometry analyses were carried out using Osteomeasure Software (OsteoMetrics, Inc., Decatur, GA). The area for measurement on the alveolar bone was identified as a square parallel to the sagittal axis of the disto-buccal root with a width that was half of the average width of the disto-buccal root and

the length extending from the bifurcation to the end of the apex. Osteoclast surface areas were determined as a surface of active osteoclasts (2+ nuclei) touching the alveolar bone and then divided by total bone surface per defined area.

Odontoclasts, were considered as TRAP-positive multinucleated cells (2+ nuclei) on the dentin surface and were counted on the mesial surface of the disto-buccal root in the line starting from the bifurcation to the end of the apex of the distobuccal root.

Statistics

Statistical analyses were carried out using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). Statistical significance of differences among means was determined by non-parametric, unpaired t-tests or non-parametric One-way ANOVA test with a Bonferroni post-test. Significance was accorded when $p < 0.05$.

Chapter IV. Results

During the course of the study one of the rats was excluded due to the loss of the orthodontic appliance.

The effect of Corticision on tooth movement

To assess if corticision has an effect on the rate of tooth movement, the distance between the first and second molars was measured in all the rats. Table

1 shows the mean \pm SD tooth movement of all the groups measured by feeler gauge. Statistically significant differences in the amount of tooth movement between the four groups were assessed by 1-way ANOVA test with a Bonferroni post-hoc test ($p < .05$) (Figure 4). Statistical significance was found only between the two corticision group and all other groups. Although the one corticision group showed increased amount of tooth movement compared to the control group this difference was not statistically significant.

Table 1. Intermolar distances measured by feeler gauge.

Control M1-M2 (mm)	1 Corticision M1-M2 (mm)	2 Corticision M1-M2 (mm)	Gingival M1-M2 (mm)
0.15	0.43	0.70	0.20
0.24	0.27	0.58	0.21
0.25	0.48	0.75	0.25
0.31	0.52	0.57	0.41
0.34	0.51	0.55	0.38
0.53	0.52	0.50	0.37
0.27	0.38	0.48	0.40
0.20	0.27		0.20
0.286 \pm 0.115 (mean \pm SD)	0.422 \pm 0.105 (mean \pm SD)	0.59 \pm 0.1 (mean \pm SD)	0.302 \pm 0.095 (mean \pm SD)

The effect of corticision on osteoclasts

To determine the osteoclastic activity, the number of osteoclasts was counted and the osteoclast surface area was measured in all the rats.

Group 1C showed a slight increase in the number of the osteoclasts (10.8 ± 5.0) but this difference was not statistically significant. The means for the other groups were control: 9.8 ± 4.0 , 2C: 10.1 ± 3.2 and the gingival group was 8.6 ± 2.5 (Fig 5). The osteoclast surface area showed a slight increase in the control group 9.9 ± 4.1 but this difference was not significant as well (1C: 7.6 ± 3.3 ; 2C: 8.5 ± 3.4 ; Gingival: 8.4 ± 3.6) (Fig 6).

The effect of corticision on odontoclasts

To measure the effect of corticision on the differentiation of odontoclasts, these cells were counted on the mesial root surface area of the distal root of the maxillary left first molar. The 2C group showed a slight decrease in the number of odontoclasts 2.7 ± 4.2 , but was not statistically significant (Control: 3.3 ± 2.1 ; 1C: 3.1 ± 2.6 ; Gingival: 3.6 ± 4.3) (Fig 7).

Corticision and the RANKL activity

Immunohistochemistry for RANKL activity was performed in all the groups. RANKL positive cells were detected in the bone marrow, some osteocytes within the alveolar bone, some cells surrounding the blood vessels and some osteoclasts. Very low RANKL activity was detected in the osteoclasts and the odontoclasts on the compression side of the PDL in all groups.

No evident difference was detected among the groups (Fig 8).

Chapter V. Discussion

Orthodontic treatment usually requires approximately two years of intervention to full completion which increases the risk of caries, periodontal disease and root resorption. Previously, different methods have been utilized to induce the RAP in order to shorten the treatment time. Many of these approaches were limited due to their invasive nature. One should also consider that the RAP is a transient process and it is logical to find a method which can be reutilized during the course of orthodontic tooth movement.

In this study, corticision was performed near the palatal surface of the maxillary first molar to examine its effects on orthodontic tooth movement. The health of the rats was not affected by the anesthesia, orthodontic appliance or the corticision procedure.

A tooth movement model in rats that utilizes NiTi coil springs to deliver a constant mesializing force on the maxillary first molar was used. Ren et al.[41] described this method as an ideal rat tooth movement model.

Based on the observations in this study, the first hypothesis that there would be greater orthodontic tooth movement in the corticision group was supported. The results showed that two corticisions significantly increase the amount of tooth movement compared to the other groups. This difference is in agreement with the findings of other studies that showed injury to the alveolar

bone increases the amount of tooth movement [33, 50, 51]. In 2008, Sebaoun et al. [18] demonstrated that selective alveolar decortication in rats increases the alveolar bone turnover rate. Likewise, Lino et al.[50] showed that corticotomy in dogs increases the rate of orthodontic tooth movement for at least 2 weeks.

Our results showed that the amount of tooth movement was almost doubled in the two corticision group compared to the control group. Some of the previous studies have shown the same ratio of increase in the amount of tooth movement.[32, 52, 53]. Table 2 shows the list of selected studies from the literature analyzing the effect of alveolar bone injury on OTM. Teixeira et al.[32] showed that tooth movement was doubled by applying small perforations in the alveolar bone in a rat model. Similarly, Moastafa et al. [52] illustrated that performing corticotomy almost doubles the rate of tooth movement in dogs.

From a translational research point of view, Aboul-Ela et al.[53] recently conducted a clinical trial in human subjects and showed that using corticotomy for canine retraction significantly increases the rate of tooth movement. This effect was also almost twice that of the control side during the first two months and almost become the same as the control group within 4 months.

Previously, Frost suggested that increasing the amount of injury to the bone may generate a greater RAP effect [28]. In a foxhound animal model Cohen et al.[54] also demonstrated that by increasing the surgical trauma to the alveolar bone the rate of tooth movement would be faster due to the increase in RAP. This could explain why we observed more tooth movement in the two corticision group compared to the other groups. In foxhounds, Sanjideh et al.[33]

showed that performing a second corticotomy maintains the higher rates of tooth movement for a longer period of time. However, the two corticotomy group showed only a marginal total increase in the amount of movement compared to the one corticotomy group. The authors attributed this effect partially to the timing of the procedure. They also suggested that they might have observed a more pronounced effect if they had increased the time gap between the first and second corticotomy procedures to allow the effect of the first to diminish. A possible explanation for the fact that our results show a significant increase in tooth movement when comparing two to one corticision is the different animal model and method of injury was utilized.

In this study, one corticision also increased the amount of tooth movement compared to the control group, but this difference was not statistically significant. This can be due to the fact that orthodontic tooth movement by itself also initiates RAP[55]; therefore, the effect of one corticision might not be sufficient to significantly increase the injury to the bone. Adding the gingival cut to the OTM did not have any significant effect on the amount of tooth movement. This result is also in line with the results of Teixeira et al.[32] who showed that adding a gingival flap to the OTM without any alveolar injury did not have any significant effect on the amount of tooth movement. The second hypothesis that corticision would increase osteoclastogenesis, was not supported. Previous studies have shown that after injury to the bone, there is a catabolic stage characterized by an increase in osteoclast number and activity, followed by an anabolic phase[31, 33, 50, 51]. In this study, although tooth movement was significantly increased in the

two corticision group, after two weeks there was no difference between the number of osteoclasts, osteoclast surface area or RANKL expression between the four groups. This can be due to the fact that the peak catabolic activity had occurred earlier during the experiment, and had returned to its baseline value after two weeks. In a recent study, Baloul *et al.* [31] demonstrated that selective alveolar decortications in a rat model increase the RNA expression of markers of osteoclastic cells and key osteoclast regulators, indicating osteoclastogenesis. However, some of these values reached their maximum level during the first week and declined to the original level after two weeks.

In 2009, Kim *et al.* [19] introduced corticision as a method to manipulate the alveolar bone without reflecting a soft tissue flap. The results of this study confirms their findings, albeit in a different animal model. In the current study, the authors decided to perform the corticision procedure less frequently. This difference was partially due to the fact that the current study is in a different animal model with different bone quality.

In their study, Kim *et al.* [20] also illustrated that using corticision results in extensive direct resorption of bundle bone with less hyalinization and more rapid removal of hyalinized tissue. In the current study, the high force (60g) was chosen to produce more hyalinization which could magnify the effect of corticision upon removal of the hyalinization tissue. Further studies need to be done to analyze the effect of corticision with lighter force application as well.

The length of our study was two weeks. This was based on previous experience from a study in the Division of Orthodontics[56], during which it was

observed that increasing time from two to four weeks could potentially decrease the accuracy of the measurement due to: (i) increased chances of bond failure between the tooth surface and the appliance/wire and (ii) spring deactivation. In this earlier study, some animals even showed a decrease in the amount of tooth movement from two to four weeks.

One of the limitations of this study was measuring the histological factors only after two weeks. As most of the RAP effect is transitory, it would have been better to do a time course study.

Prior to applying this method in a clinical setting, we also first need to conduct further studies with: (i) a larger sample size, (ii) different force levels and (iii) a more in depth histological evaluation in order to better understand the underlying mechanisms involved in the bone manipulation. Knowing that OTM can also induce RAP, it would be appealing to perform only one corticision a week after appliance placement to see if that can also increase the rate of tooth movement significantly.

Chapter VI. Summary and Conclusions

Corticision can be a promising approach to accelerate tooth movement. However, it is important to use caution in applying this rodent data to humans due to the different force magnitude and also the different quality of bone. Rats are the most commonly used animal models; however, there are some structural differences between rat and human alveolar bone. In the rat, bone is denser, exhibits no osteons and its bone plates are void of marrow spaces[57]. Future *in vivo* studies need to be done in order to prove these effects in humans as well.

Chapter VII: Figures



Fig 1. Placement of the closed coil spring in an *In vivo* rat tooth movement model. Spring has been activated between the maxillary left first molar to incisors.

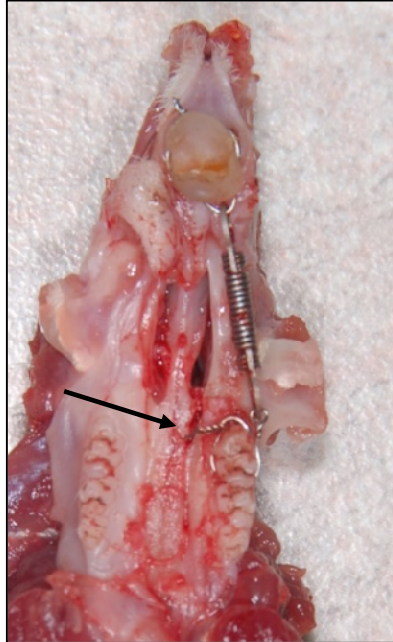


Fig 2. Localization of corticision in relation of maxillary first molar. Dissected rat maxilla, with a spring being activated from the maxillary left first molar to the incisor. The arrow points to the corticision cut in the alveolar bone.

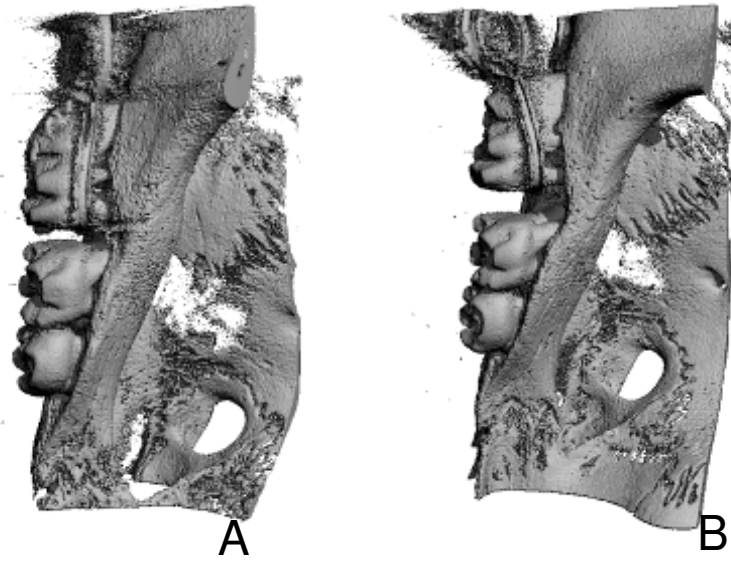


Figure 3. Micro-CT image of the dissected maxilla following OTM. An example of micro-CT on a dissected maxilla after 2 weeks of tooth movement. **A.** Control group. **B.** Two corticision group.

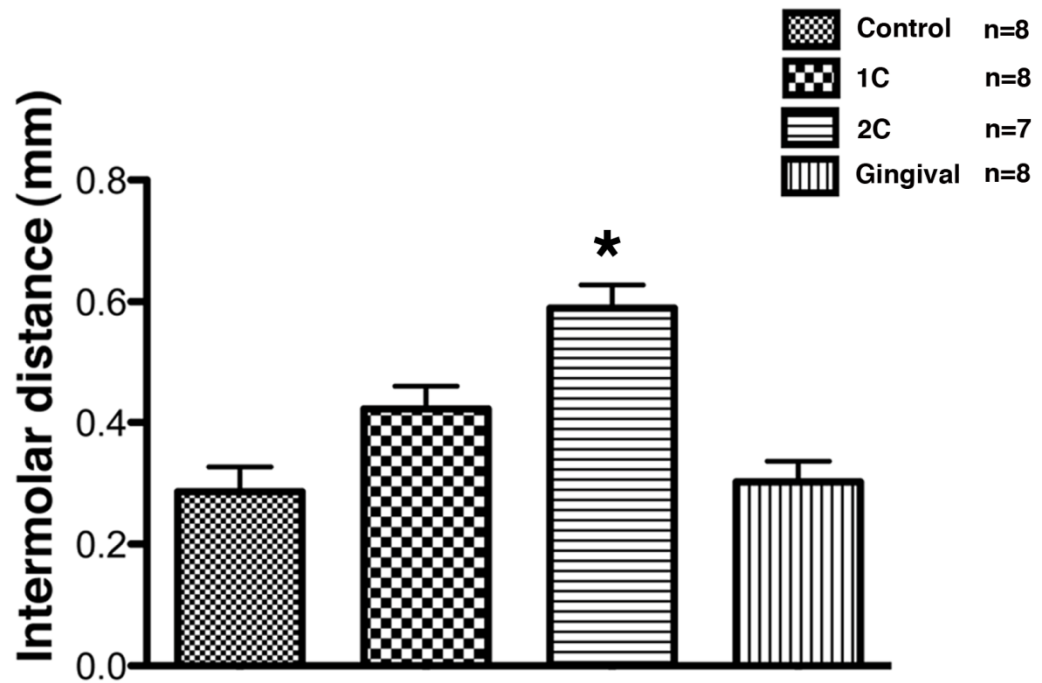


Fig 4. Evaluation of intermolar distance following OTM.

Graph showing the intermolar distances from four different groups at day 14. Each value represents the mean \pm SEM. Significantly more tooth movement was found in the 2C group compared to the other groups. (ANOVA, $p < 0.0001$).

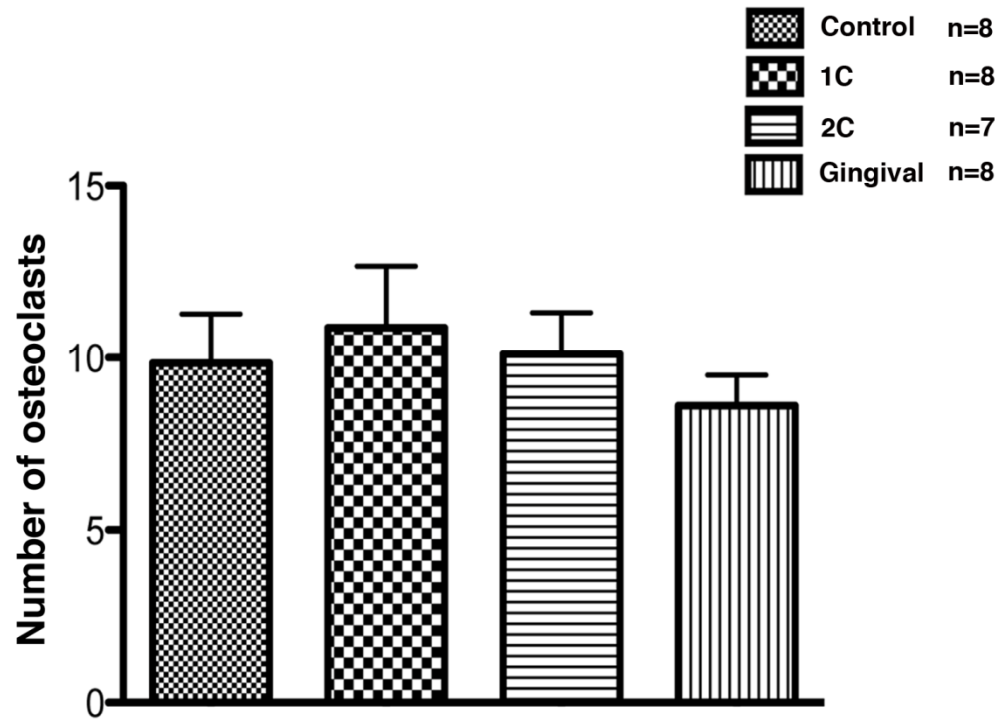


Fig 5. Histological quantification of active osteoclasts on the alveolar bone.

Number of osteoclasts were quantified on the compression side of the left maxillary molars. An active osteoclast was considered as a TRAP positive, multinucleated cell (2+ nuclei) touching the bone surface. No statistical difference was observed between the groups (1-way ANOVA, $p = 0.7024$). Each value represents the mean \pm SEM.

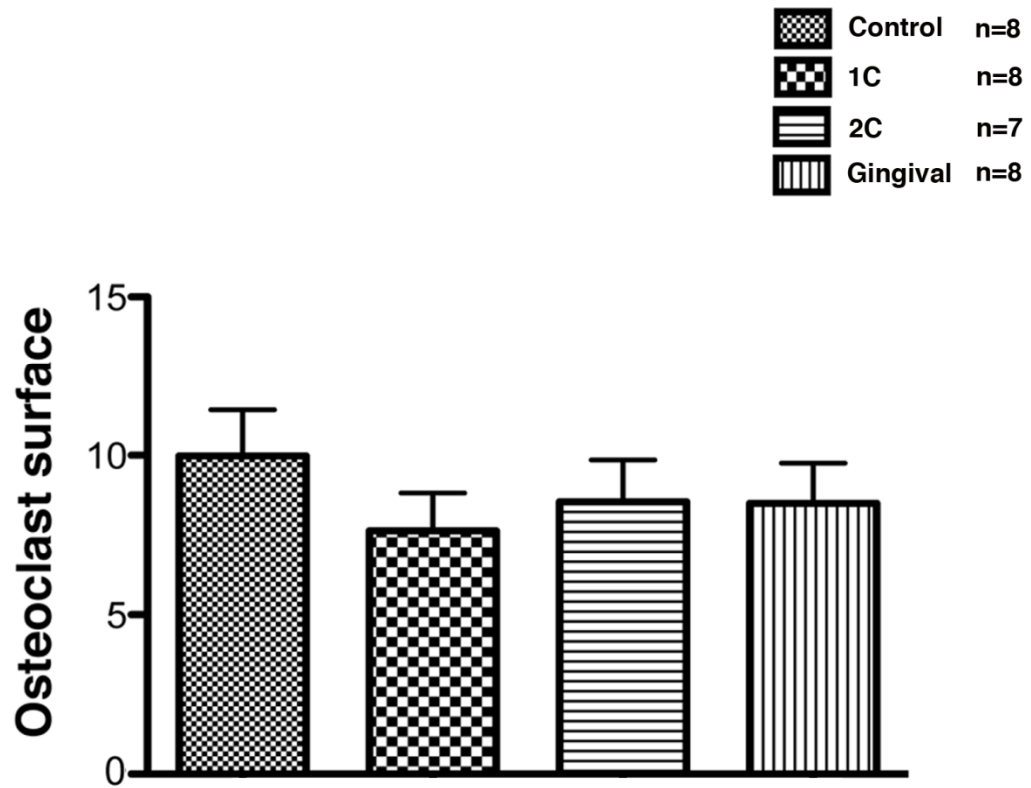


Fig 6. Graph representing the osteoclast surface parameter.

Osteoclast surface was calculated as a surface of active osteoclast touching the bone surface divided by the total bone surface per defined area. No statistical difference was observed among the groups (1-way ANOVA, $p= 0.095$). Each value represents the mean \pm SEM.

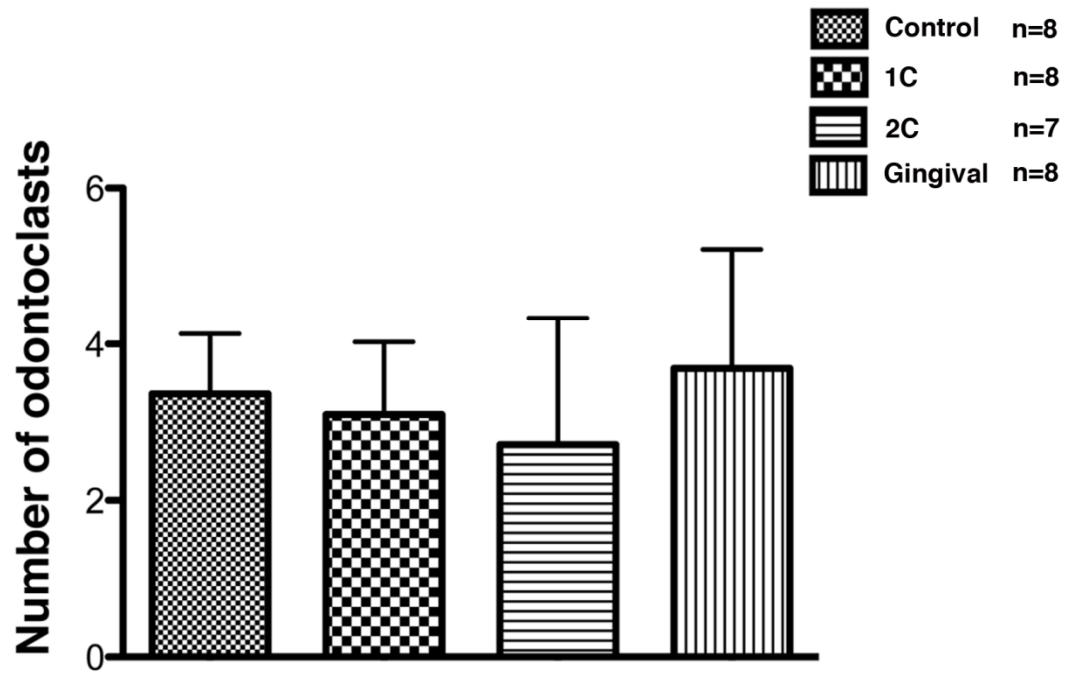


Fig 7. Histological quantification of active odontoclasts.

The number of odontoclasts were quantified on the mesial surface of the disto-buccal root on the compression side of the left maxillary molars. An active odontoclast was considered as a TRAP positive, multinucleated cell (2+ nuclei) touching the root surface. No statistical difference was observed between the groups (1-way ANOVA, $p=0.956$). Each value represents the mean \pm SEM.

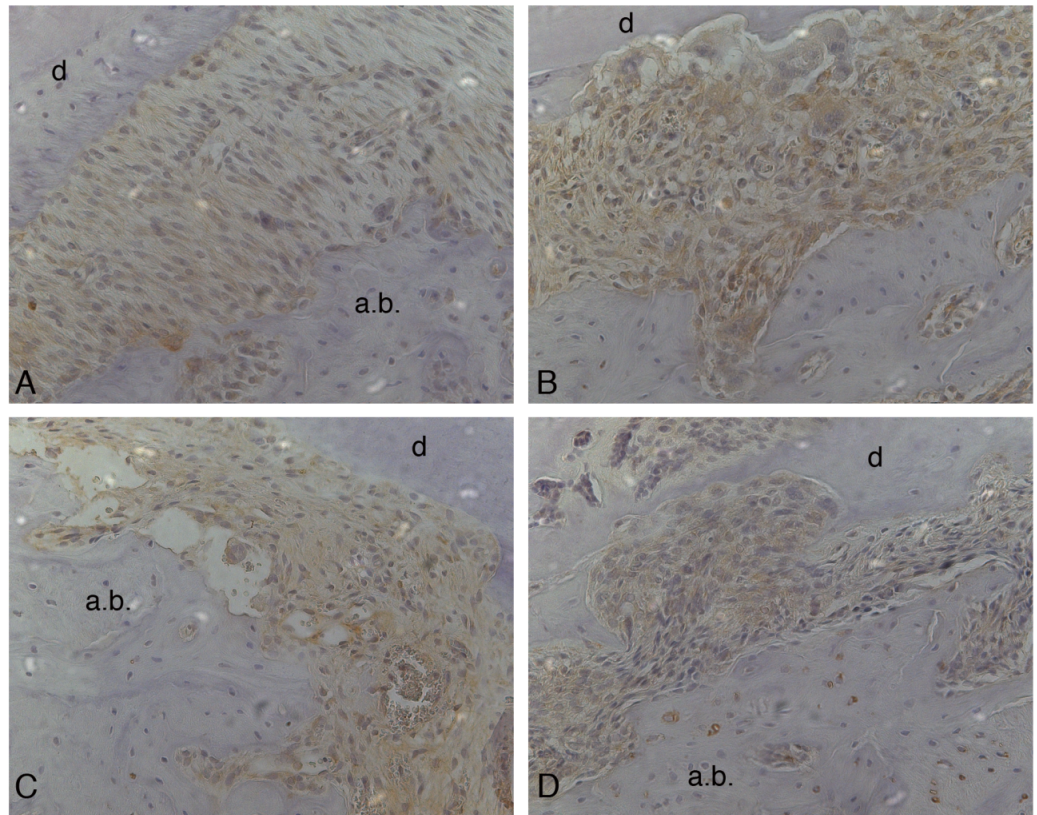


Fig 8. Detection of RANKL expression by Immunohistochemistry.

RANKL expression was evaluated on the mesial surface of the distal root of left maxillary first molar after two weeks of orthodontic tooth movement: A, Control group; B, I C group; C, 2C group; D, gingival group. a.b., alveolar bone; d, dentin.

Table 2. List of selected studies from the literature analyzing the effect of alveolar bone injury on OTM. ^a: Periodontal distraction. ^b: Dentoalveolar distraction.

Study	Subjects	Procedure	Force	Time	OTM (mm) Experimental Group
Baloul et al.[31]	Rat	Selective Alveolar Decortication	25g	42days	2.29
Aboul-Ela et al.[53]	Human	Corticotomy	150g	4 months	5.68
Teixeira et al.[32]	Rat	3 small perforations of cortical plate	50 g	28 days	0.62
Lee et al.[58]	Rat	Corticotomy/ Osteotomy	100g	21 days	Osteotomy: 0.44 Corticotomy: 0.33
Cohen et al.[54]	Dog	Periodontal distraction/ Dentoalveolar distraction	Heavy Distraction force	15 days	^a PD: 2.9 ^b DD: 1.8
Sanjideh et al.[59]	Dog	Corticotomy 2 times	200g	56 days	2.4
Mostafa et al. [52]	Dog	Corticotomy	400g	5 weeks	4.67
Kim et al.[19]	Dog	Corticision	150g	8 weeks	4.61
Lino et al.[50]	Dog	Corticotomy	500g	4 weeks	2.0
Current study	Rat	Corticision 2 times	60 g	2 weeks	0.59

Table 2. Continued

Study	OTM (mm) Experimental Group	OTM (mm) Control Group	OTM Experimental / OTM Control 2 weeks	OTM (mm) Experimental /OTM (mm) Control Total	OTM Experimental / OTM Control 2 weeks
Baloul et al.[31]	2.29	2.3	1.0/1.1	0.99	0.9
Aboul-Ela et al.[53]	5.68	3.39	0.94/0.37	1.67	2.54
Teixeira et al.[32]	0.62	0.29		2.13	
Lee et al.[58]	Osteotomy: 0.44 Corticotomy: 0.33	Control: 0.3		Osteotomy: 1.46 Corticotomy: 1.1	
Cohen et al.[54]	^a PD: 2.9 ^b DD: 1.8	No control			
Sanjideh et al.[59]	2.4	1.3	0.5/0.25	1.84	2
Mostafa et al. [52]	4.67	2.33	3.5/1.5	2	2.33
Kim et al.[19]	4.61	1.23	2.0/0.25	3.74	8
Lino et al.[50]	2.0	1.0	1.1/0.5	2	2.2
Current study	0.59	0.28	0.59/0.28	2.1	2.1

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