EFFECTS OF LOCAL ADMINISTRATION OF KETOROLAC TROMETHAMINE, A NONSTEROIDAL ANTI-INFLAMMATORY DRUG, ON ORTHODONTIC TOOTH MOVEMENT IN RATS

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A thesis submitted to the faculty of The University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Department of Orthodontics in the School of Dentistry.

Chapel Hill

2006

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ABSTRACT

EROL AGI: Effects of local administration of ketorolac tromethamine, a nonsteroidal anti-inflammatory drug, on orthodontic tooth movement in rats.
(Under the direction of Dr. William R. Proffit)

Tooth movement rates can be inhibited by prostaglandin inhibitors acting systemically. We investigated the locally delivered effects of ketorolac tromethamine (KT), a potent nonsteroidal anti-inflammatory drug (NSAID), on orthodontic tooth movements in twelve Wistar rats. U-shaped expansion springs were positioned between upper first molars exerting equal and reciprocal, laterally directed forces for seventeen days. Every fourth day 1.5 mg of KT in solution was delivered around one molar and saline solution was injected adjacent to the contralateral molar. Subsequent to a wash-out period lasting fifteen days of continued expansion, the appliances were removed, the teeth allowed to relapse for thirteen days while the same schedule was maintained for the NSAID delivery. Combining the data from all the teeth that received KT and comparing those with the control data, no significant differences were seen with their movements during the expansion or relapse phases.

To Francesca and Filippo

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to all those who contributed to the completion of this thesis:

My research advisor, Dr. William R. Proffit, your profound knowledge, continuous guidance and immeasurable support have made the difference. I thank you.

My committee members, Dr. Ray Williams and Dr. Steven Offenbacher, thank you for your helpful advice and for your generous permission to utilize your valuable research resources.

Dr. Estelle Riche and Dr. David Barrow your insight, advice and help made working with the rats a possibility and an enjoyable experience.

Mr. Robert Paterson, your expertise in mathematics is the finest, thank you.

Mr. Jermaine Fuller, Mr. Jason Pollei, and Ms. Silvana Barros, for your many tireless hours of research assistance, thank you.

Mr. Wallace Ambrose, for your assistance and support in using the electron microscopy lab, thank you.

Dr. Ceib Phillips and Ms. Debbie Price for your help with the statistics, thank you.

Mr. Mike Wile, for your donation of the Dentsply-GAC, International, Inc. (Bohemia, NY) nickel titanium wires for this research project, thank you.

Francesca Ronca and my son, Filippo Agi, you are both my inspiration. I thank you for supporting me and for the sacrifices you made.

This work was supported in part by the University of North Carolina's Orthodontic Fund.

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LIST OF ABBREVIATIONS

cAMP	cyclic adenosine monophosphate
COX	cyclooxygenase enzyme
GCF	gingival crevicular fluid
IACUC	Institutional animal care and use committee
IL-1	interleukin-1
IP	intraperitoneal
IV	intravenous
KT	ketorolac tromethamine
NIH	National Institutes of Health
NiTi	nickel titanium
NSAID	nonsteroidal anti-inflammatory drug
PDL	periodontal ligament
PG	prostaglandin
PGE	prostaglandin E
PO	peroral
Pt.	point
RP	reference point
SEM	scanning electron microscope
SS	stainless steel
TNF	tumor necrosis factor
UL	upper left

UR	upper right
W/V	weight by volume

I. INTRODUCTION

A biologic principle in orthodontic tooth movement is thatprolonged mechanical pressure on teeth results in remodeling of periodontal structures, which includes the periodontal ligament (PDL) and alveolar bone (Proffit, 2000). A condition for the remodeling activities, and ultimately tooth displacement, is the occurrence of an inflammatory response (Vandevska-Radunovic, 1999). During orthodontic treatment, low-intensity and continuous forces are placed on teeth, causing physical distortion beyond the bio-adaptability or bio-elasticity of the PDL fibers (Storey, 1973). The pressure and tension within the PDL, causing a shift in tooth position, promotes, within a few hours, a cascade of events leading to an inflammatory response (Davidovitch *et al,* 1988). T he change in the perfusion dynamics in the PDL is followed by the cellular production and release of inflammatory mediators within the periodontium, that ultimately allows bone remodeling and tooth translation (Proffit, 2000).

Acute inflammation is characterized by redness, heat, swelling, pain and loss of function. These macroscopic changes are produced by a progression of vascular events, such as vasodilation and increased permeability of small blood vessels within a damaged area, the exudation of fluids, and leukocyte migration into the extravascular spaces. These vascular changes are accompanied by the release of inflammatory mediators, namely histamine, prostaglandins, leukotrienes and cytokines (Stephenson, 1992).

The cardinal signs of inflammation are often observed during orthodontic treatment, such as redness and swelling of the gingiva (Vandevska-Radunovic, 1999). Pain that begins within several hours after application of orthodontic forces and may last for a few days is a common experience in nearly all orthodontic patients. As pain leads to impaired function, biting and chewing become more uncomfortable, which leads to some degree of functional loss (Scheurer *et al.,* 1996).

For more direct evidence of inflammatory alterations in the PDL during orthodontic treatment, studies were conducted on animal subjects (reviewed in Vandevska-Radunovic, 1999). Changes in the vasculature of the periodontium were observed in monkeys and dogs during orthodontic tooth movements. Within minutes of force application, teeth moved within the PDL space compressing towards the alveolar wall. In the compressed or pressure area, PDL space was narrowed and blood vessels were partially or completelyoccluded. On the tension side, PDL space was widened and blood vessels were dilated and engorged with blood (Khouw and Goldhaber, 1970). After three days of continuous force applied to the teeth, the tension side showed osteoblasts lining the bone, while the pressure side still had no evidence of bone resorption (Khouw and Goldhaber, 1970). Bone deposition and resorption started around the seventh day of applied forces. Ligaments on the tension side remained stretched and clear evidence of new bone formation was observed. The pressure side had an increase in vascularity with many new capillary loops in close proximity to the osteoclast lined alveolar bone undergoing active resorption (Khouw and Goldhaber, 1970).

Other studies provided evidence that changes in the vascularization within the PDL were directly correlated to the start of the inflammatory process. Vandevska-Radunovic *et al., (*1994) reported that vasodilation occurred within minutes on the tension side of the PDL

with increased blood flow and fluid exudates from dilated blood vessels. Furthermore, an immediate extra-vascular movement of leukocytes into the tissue spaces was observed on the pressure side, which was characterized by a decrease in PDL space and partially occluded blood vessels (Iida *et al*, 1996). Within days of sustained pressure, several lines of inflammatory cells, such as monocytes and polymorphonuclear leukocytes, known to differentiate into macrophages and osteoclasts, were observed throughout the periodontium (Iida *et al.,* 1992).

For teeth to move through bone, both soft and hard tissues must be remodeled. Breakdown of bone by osteoclasts occurs in the compression side, the area toward which the tooth is moving. On the other hand, osteoblasts deposit newly formed bone in the tension side, the area away from which the tooth is moving, while the fibroblasts contribute to the turnover of the collagen matrix of the PDL (Proffit, 2000). Studies by Kvam (1972), Smith and Roberts (1980), McCulloch and Melcher (1983) and McCulloch *et al,* (1987) showed orthodontic forces promoted the mitotic activity of resident fibroblasts, osteoclasts and osteoblasts. Since the entire vascular network was increased, it facilitated the migration of precursor cells from the immediate marrow spaces towards the active remodeling sites. These changes in the cellular activities and the consequent inflammatory process were found to be controlled and sustained by various inflammatory mediators detected within the periodontium (Vandevska-Radunovic, 1999). During acute episodes of inflammation, there were increased levels of prostaglandins (PGs), cyclic adenosine monophosphate (cAMP), interleukin-1 (IL-1), and tumor necrosis factor (TNF) (reviewed in Yamaguchi and Kasai, 2005).

IL-1 and TNF are pro-inflammatory cytokines known to promote acute and chronic inflammation, possibly by inducing the expression of specific pro-inflammatory proteins, such as the monocyte chemoattractant protein-1 from fibroblasts (Dinarello, 1991; Ozaki *et al.,* 1996). Both IL-1 and TNF levels increased in the PDL and alveolar bone in cats during orthodontic tooth movement, suggesting they play a role in bone remodeling events (Davidovitch *et al*, 1988 ; Davidovitch, 1991; Saito *et al.,* 1991). In accord with these data, other investigators observed a synergistic increase in the levels of IL-1 and PGE_2 in the gingival crevicular fluid (GCF), following sustained mechanical stress (Grieve *et al*., 1994; Lee *et al*., 2004)., again suggesting these molecules may play a role in bone remodeling events.

Prostaglandins (PGs), metabolites of arachidonic acid metabolism, are local hormone-like agents produced by many cells, including osteoblasts, within seconds of cell injury. In response to mechanical or chemical stimuli, arachidonic acid is produced from the plasma membrane phospholipids and can be converted to PGs by a family of cyclooxygenase enzymes (COXs) (Offenbacher *et al.*, 1981). Among the members of the COX family, both the constitutive enzyme COX-1 and the inducible form COX-2, responsive to cytokines, bacterial lipopolysaccharides and growth factors, contribute to increase the PGE_2 levels (Murakami and Kudo, 2004; Gordon *et al*., 2002; Yamaguchi and Kasai, 2005).

Among the PGs produced, PGE_2 is known to increase vascular permeability and chemotactic properties of the white blood cells. PGE_2, which is produced in the bone mainly by osteoblasts, stimulates both bone formation and resorption and has been implicated in the pathogenesis of certain conditions such as periodontitis and osteomyelitis, which are associated with bacterial induced bone loss. Furthermore, PGE_2 was shown to facilitate the

increased formation of osteoclasts, thereby escalating bone and root resorption (Yamaguchi and Kasai, 2005; Boekenoogen *et al.,* 1996). Further implicating PGE_2 as an important mediator of bone resorption, increased levels in the periodontal tissues, as measured in the GCF, were found to correlate with periodontal bone resorption and the progression of periodontal disease (Offenbacher *et al,* 1981).

In orthodontic treatment the rate of bone resorption and formation are factors in determining the velocity of tooth movement. This knowledge led investigators to study whether PG levels could be altered to influence the pace of tooth movement. In fact, PGs were successfully used to increase the speed of tooth movement. Injections of exogenous PGE_1 and PGE_2 adjacent to investigative teeth increased their tempo of up to two times as compared to the control teeth in rats, monkeys and humans (Kale *et al*., 2004; Leiker *et al*., 1995; Yamasaki, *et al.,* 1982, 1984). However, a significant amount of root resorption was observed over a 2-week period in rats that received local injections of exogenous PGE_2 (Boekenoogen *et al*., 1996). PGE_2 was also found in periodontal cysts, dentigerous cysts, keratocysts and ameloblastomas, lesions that are associated with bone and root resorption (Harris *et al*., 1973). Injections of exogenous PGs may increase rates of tooth movement, but they also have the potential to introduce extremely negative inflammatory reactions if given the chance.

On the other hand, the inhibition of tooth movement by using PG inhibitors could be advantageous in many clinical orthodontic situations by increasing the anchorage value of teeth. Anchorage in orthodontics is defined as the resistance to unwanted tooth movement. Teeth that can maintain their position in a dental arch by their increased resistance to movement can help absorb the reactive force of moving other teeth (Proffit, 2000).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are known to be non-specific COX inhibitors. They act as anti-inflammatory drugs by both inhibiting the COX enzymes expression and their catalytic activities, thus decreasing the overall PG production (Murakami and Kudo, 2004). Yamasaki *et al.,* (1980) showed that indomethacin, a NSAID, inhibited the number of osteoclasts around teeth that received the drug injection. More recently, indomethacin was shown to decrease the rate of bone turnover in miniature pigs (Giunta *et al*, 1995) and total tooth movement in rats and cats (Mohammed *et al.*, 1989; Chumbley and Tuncay, 1986).

In other studies, flurbiprofen, a different NSAID compound, decreased the number of osteoclasts but did not affect tooth movement rates (Sandy and Harris, 1984). Similarly, Wong *et al.,* (1992) showed that aspirin, an irreversible COX inhibitor (Sari *et al*., 2004), did not decrease the rate of tooth movement in guinea pigs.

Hoping to clarify the literature disagreement on whether an anti-inflammatory drug could decrease inflammation resulting from orthodontic pressure and thereby increase the anchorage value of teeth, we hypothesized thatthe effects of another NSAID , ketorolac tromethamine (KT), could alter tooth movement when administered locally. KT was chosen because it was found to decrease concentrations of PGE_2 in dogs (Pasloske *et al*, 1998) and to be a potent bone resorption inhibitor in humans (Allison *et al.,* 1993). Due to its strong anti-inflammatory effect, its water solubility, lack of irritation to mucosal tissue and its absence of taste, topical forms of KT have been used successfully to halt the progression of periodontal disease (Kelm *et al.,* 1996).

To test the hypothesis that KT could be used locally to inhibit tooth movement and increase the anchorage value of teeth, we set up a two-phase experiment with Wistar rats. Using a split-mouth design, the experimental plan was to create transverse expansion of the upper first molars while teeth were exposed to KT on one side and saline solution on the other. Impressions to show the position of the teeth were taken during the expansion phase of tooth movement, and again as relapse tooth movement occurred after the expansion was completed. The impressions were examined and recorded with a scanning electron microscope. Images were then digitized, so that a special software program, developed at the National Institutes of Health (NIH), could be used to calculate the relative movements of teeth.

II. MATERIALS AND METHODS

A. ANIMALS

Rats were chosen for this project because they have been used in many similar orthodontic tooth movement studies (Yamasaki*et al.,* 1980; Yamasaki, 1983; Mohammed *et al.*, 1989; Vandevska-Radunovic *et al*, 1994; Leiker *et al*, 1995; Boekenoogen *et al*, 1996; Soma *et al.*, 1999; Kyrkanides *et al.*, 2000; Kalia *et al*, 2004; Dolce *et al*, 2003). T heir dentition may be small but nonetheless large enough to allow the necessary study. Rats have been identified as a good animal model because of their skeletal adaptation to mechanical forces (Jee *et al*, 1991; Ren, *et al.*, 2004). Additionally, rats were easily obtainable, relatively inexpensive to house and feed and more could be worked on simultaneously than larger animals. Although the size of the animals made it difficult, the NSAID was injected adjacent to the teeth under investigation, preventing the drug from falling out of the sulcus as could have occurred with larger sized animals with larger gingival sulci.

The University of North Carolina at Chapel Hill's (UNCCH) Institutional Animal Care and Use Committee (IACUC) pre-application tests were successfully taken by all the participants of this project who were involved with direct animal contact. The application to conduct this investigation was approved by IACUC in Dec 2004 and again in Oct 2005 as per annual requirements. The application protocol number assigned was 04-278.0-A.

A total of 16 male Wistar rats at 6-7 weeks of age were purchased from Harlan Laboratory (Indianapolis, Indiana), provided a standard dry laboratory food, Purina RMH

3000, and allowed to drink water *ad libitum.* They were housed two per cage in a temperature- and climate-controlled room. Only male rats were ordered to eliminate the hormonal changes associated with estrus. These rats were exposed to automated cycles of twelve hours of light and twelve hours of darkness. The average weight was 260.5 grams (g) per rat on day 1 at the beginning of the experiment . The rats were randomly housed two per cage and the cages were labeled A through H. Within each cage, one rat chosen at random had its tail tattooed while the other did not. This allowed distinguishing between rats in each cage. The tattooed rat was identified as rat number one and the non-tattooed rat was labeled as number two for that particular cage. Therefore, the rats were labeled and named A1, A2, B1, B2, C1, C2, D1, D2, E1, E2, F1, F2, G1, G2, H1, H2.

B. DRUG

The NSAID used in this study was ketorolac tromethamine (KT) because it was found as a more potent inhibitor of bone resorption than other NSAIDs, such as flurbiprofen, naproxen, piroxicam or ibuprofen (Allison *et al.,* 1993). In a study by Kelm *et al.,* (1996), KT was used locally as the active ingredient in a mouth rinse and dentifrice. Their results showed the concentration of KT in the GCF was at high enough levels to inhibit PGE_2 production. In a similar study, Jeffcoat *et al.,* (1995) managed to halt the progression of periodontal bone loss using KT oral rinses better than tablets of flurbiprofen taken perorally.

KT is considered a potent NSAID and is normally prescribed for moderately severe acute pain that would otherwise require analgesia at the opioid level. It is available commercially in solution form as Toradol® IV/IM (Roche Laboratories, Basel, Switzerland). It is packaged in carpules and TORADOL® is available for intravenous or intramuscular (IM)

administration as 15 mg in 1 ml (1.5%) and 30 mg in 1 ml (3%) in sterile solution. The carpule with 60 mg in 2 ml (3%) of KT in sterile solution is available for IM administration only. The solutions contain 0.1% citric acid, 10% weight by volume (w/v) alcohol and sodium chloride in sterile water. The pH is adjusted with sodium hydroxide or hydrochloric acid, and the solutions are packaged with nitrogen. The sterile solutions are clear and slightly yellow in color. The solution used for this experiment was the IM form (Fig. 1).

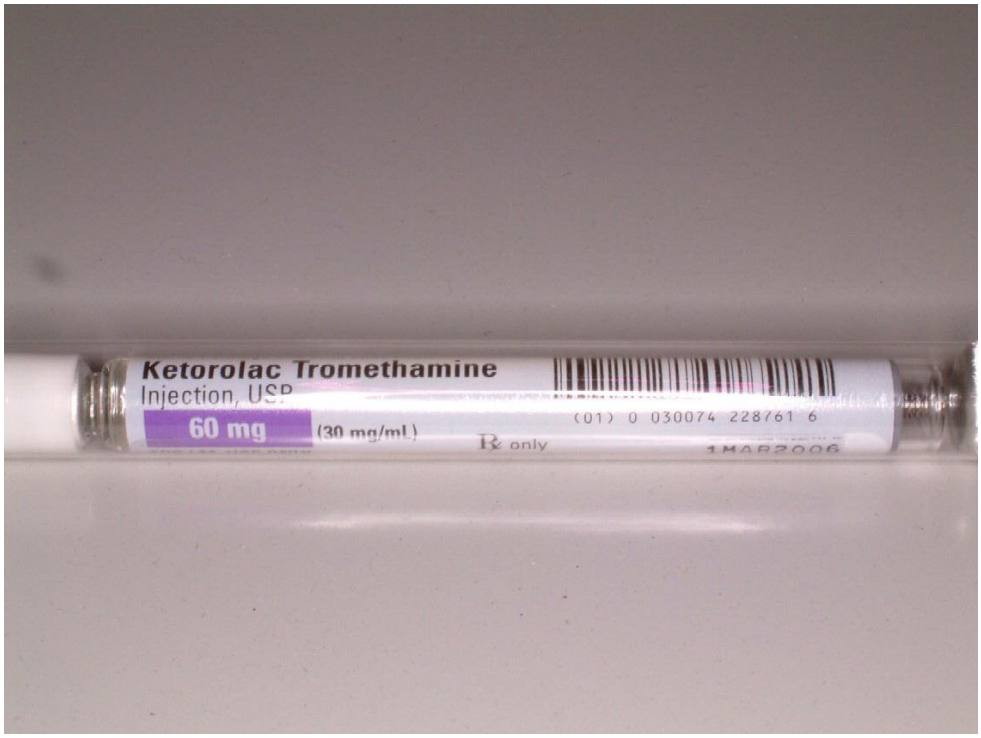

Fig. 1: Ketorolac tromethamine carpule.

Ketorolac tromethamine is a member of the pyrrolo-pyrrole group of the nonsteroidal anti-inflammatory drugs and its chemical name is (±)-5-benzoyl-2,3-dihydro-1H-

pyrrolizine-1-carboxylic acid, compounded with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1). The chemical structure is shown in Fig. 2.

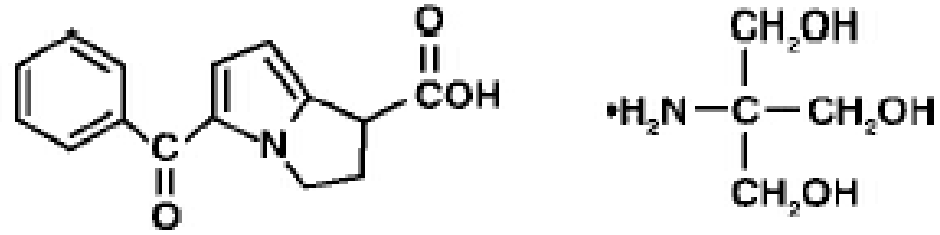

Fig. 2: Chemical structure of ketorolac tromethamine.

The investigative teeth in this study had 1.5 mg subperiosteal KT injections of the commercially available Toradol® IM solution at a concentration of 60 mg/2 ml (3% w/v). The control teeth received subperiosteal injections of 0.05 ml of 0.9% sterile saline solution (150 mM NaCl).

The anesthetic used in this study was the injectable form of a ketamine solution. The solutions and concentrations used were: Ketamine 70 mg/kg, Xylazine 4 mg/kg and 0.9% saline solution (150 mM NaCl). This anesthetic solution was administered by intraperitoneal (IP) injections. Within several minutes the rats were immobilized and anesthetized. The effects of the anesthetic normally lasted between 45 and 60 minutes per rat. After the required work on the rats was completed during each session, the rats were placed into the recovery position (Fig. 3). Once awake and alert, they were then placed back into their cages.

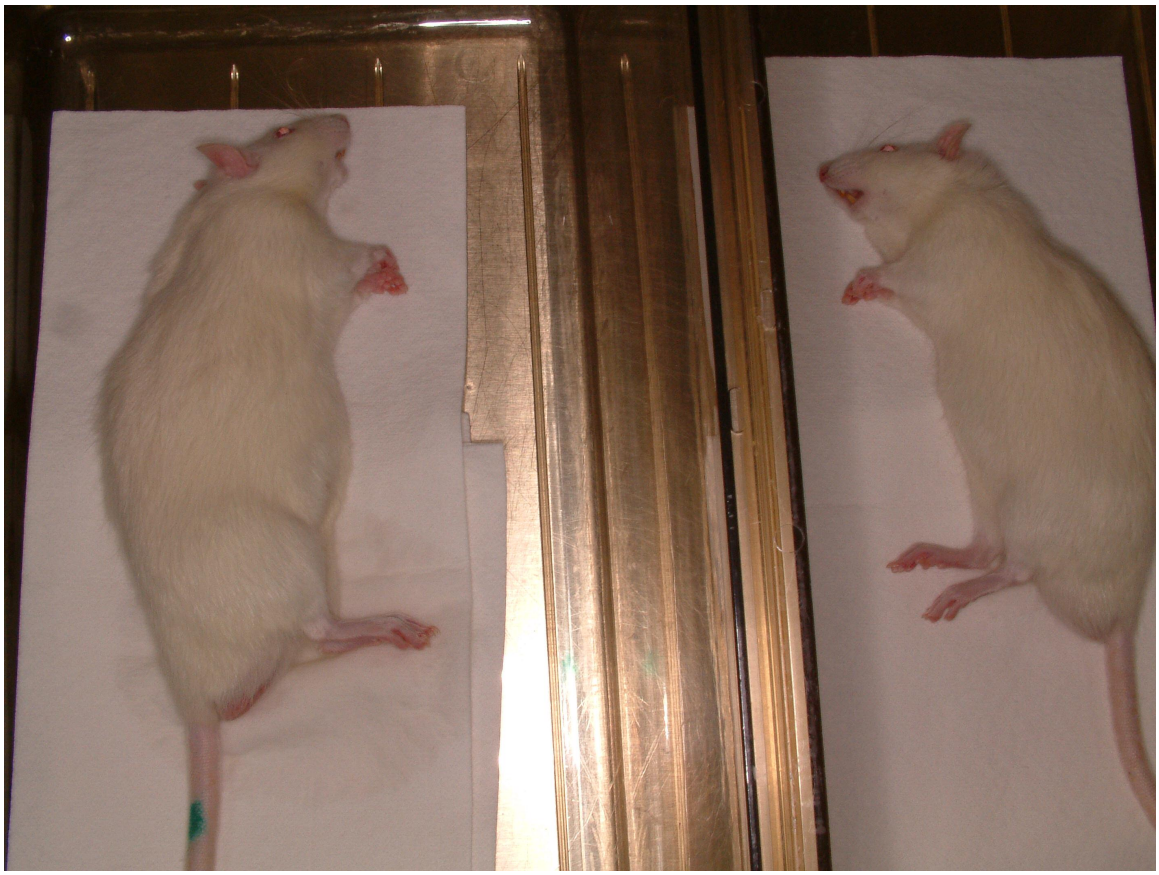

Fig. 3: Two rats in the recovery position after anesthesia.

During the pilot study, isoflurane inhalation was evaluated as the anesthetizing agent. Isoflurane was an effective anesthetic, however the management and manipulation of the rats while simultaneously managing the nasal canula was deemed impossible when working alone. Therefore, the inhalation technique was abandoned and the injectable form of the ketamine solution was used throughout the study.

C. ORTHODONTIC APPLIANCE

A pilot study using four Wistar rats was conducted in preparation for this project. In an attempt to replicate the appliance design and protocols as described by Igarashi *et al,*

(1994), we fabricated and successfully placed 0.012" nickel titanium (NiTi) orthodontic springs intraorally in these rats. However, we found these springs were not retained in their mouths between operative sessions. We were not able to reproduce the same retention as Igarashi *et al,* (1994) .

Springs of the same design were made using 0.010" stainlesss teel (SS) wires. Their improved contour and fit helped increase the retention of the appliances. The 0.010" SS springs were retained in the mouths of the rats at each of the pilot sessions and these findings lead us to use the stainless steel springs during the actual experiment. None of the tooth movement data from the pilot study was evaluated.

The springs were shaped into a U pattern with small projections at the ends to allow for improved retention interproximally between the upper first and second molars (Fig. 4). These U-shaped standard sized expansion springs were placed into each rat's mouth between the upper right (UR) and left (UL) first molars and held in the mouth by their own expansion force, which moved the UR and UL first molars laterally (Fig. 5). The dimensions were as follows: length: 5.0 mm, width: 5.5 mm and the two small projections were 1.5 mm.

The forces produced by the springs used in this study were remarkably different. Highest force levels were produced by the 0.012" SS springs per unit of activation. At a deflection of 1.5 mm, the 0.012" SS spring produced 59.3 g of force while the 0.012" NiTi spring elicited 10.3 gof force. The force -deflection curves for the three springs are represented in Fig. 6. The forces were measured using a hand-held force gauge, the Orthometer by OrthoMeasurements, a Division of Young Research & Development, Inc., (Avon, CT).

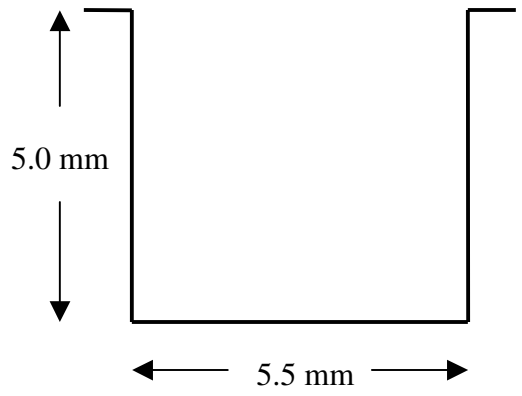

Fig. 4: Schematic of the orthodontic appliance.

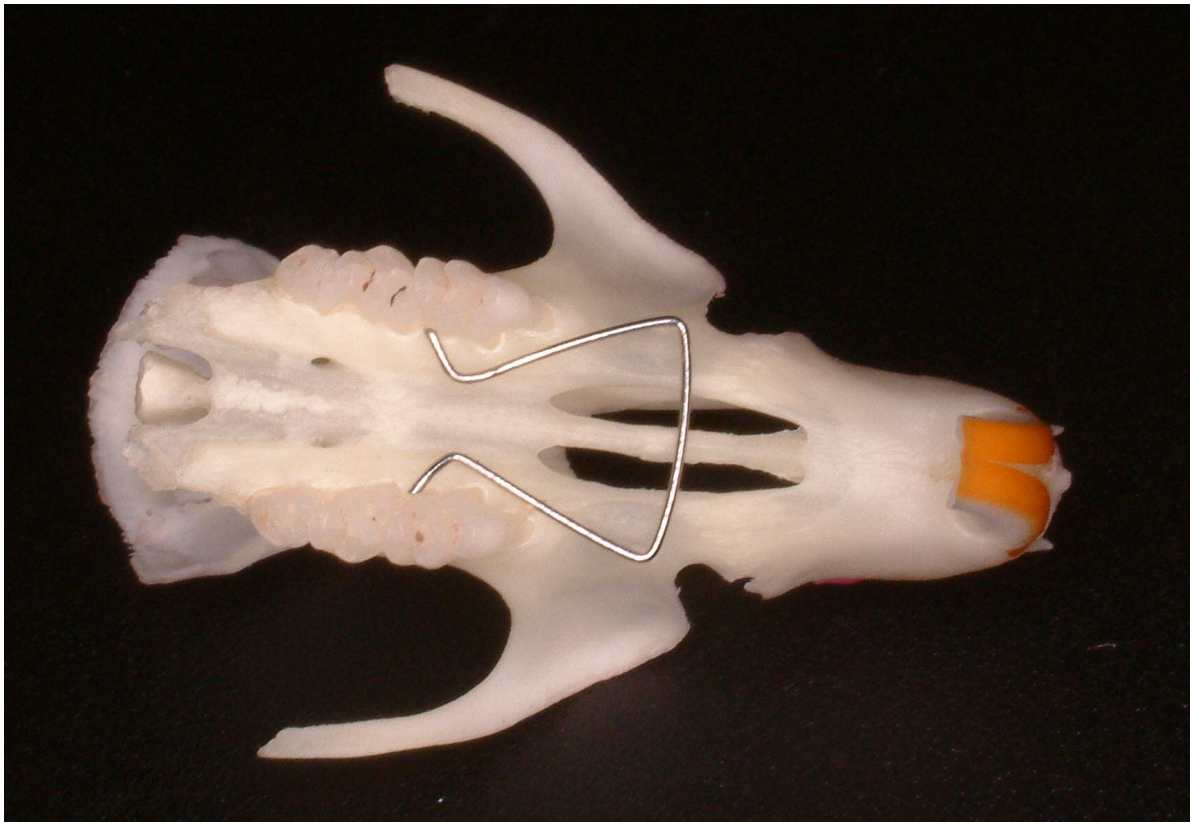

Fig. 5: 0.012” NiTi orthodontic spring in position between upper first molars of the rat maxilla.

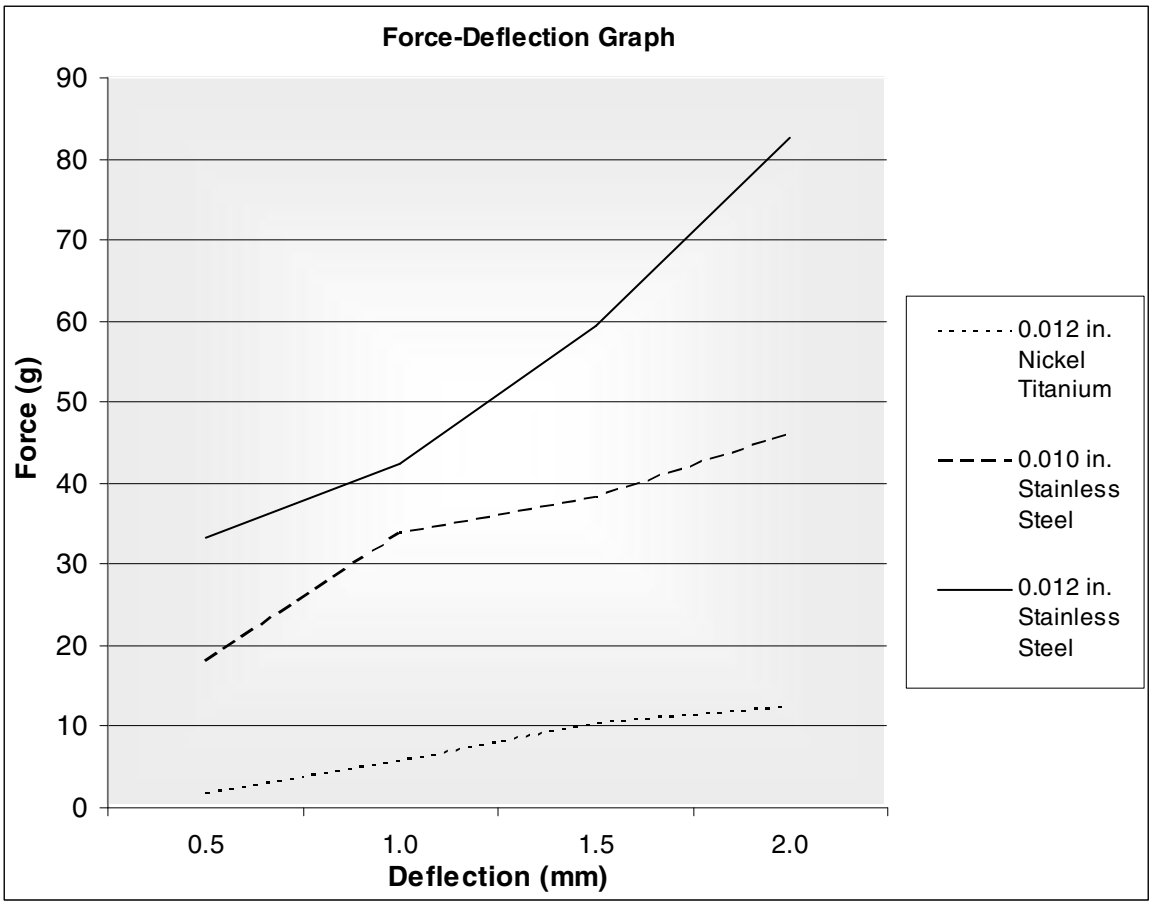

Fig. 6: Force-deflection curves for the three springs used.

D. EXPERIMENTAL PROCEDURES

1. KT Application

In each animal, 1.5 mg KT in the standardized commercially available solution was injected locally adjacent to either the UR or UL first molar. This study was performed as a split mouth design, therefore the contralateral first molar, the control tooth had 0.05 ml of 0.9 % saline solution (150 mM NaCl) administered in the same manner. The syringes used for local administration of either KT or saline were 30 gauge 0.5 inch needles (Becton,

Dickinson and Company, Franklin Lakes, NJ). By convention, the rats tagged with a tattoo on their tails and named number one of the cages they lived in, always had their upper right first molars as the site for the KT injections. Their UL first molars were their control teeth. The rats in all the cages that were labeled number two by their lack of having a tattoo always had their upper left first molars as the sight for the KT injections. Their UR first molars were their control teeth.

2. Frequency of KT Injections

General anesthesia was induced by a ketamine solution. Then every four days, under anesthesia, KT and saline were administered to the predetermined UR and UL first molars. This schedule was undertaken to prevent excess animal stress, tissue damage and animal demise by either the anesthetic or the NSAID.

3. The Expansion - Wash-out - Relapse Phases

These twelve rats underwent two phases of experiments. Sandwiched between these two phases was a period of wash-out lasting two weeks. During this wash-out time the expansive appliances remained in the mouths of the rats but no injections or impressions were made.

The first part of this project was the expansion phase. General anesthesia and administration of KT and saline were conducted every fourth day for seventeen days. This allowed for five sessions of general anesthesia, five maxillary impressions (day 1, 5, 9, 13 and 17) and four local injections of KT and saline (day 1, 5, 9 and 13) per rat. On day one a maxillary impression was taken, an orthodontic spring was positioned in each rat's mouth

and 1.5 mg of KT in solution was administered (Fig. 7). Then every four days, the rats were put under general anesthesia and the ketorolac tromethamine was administered adjacent to either the UR or UL first molars. On day 17, the rats were put under general anesthesia, impressions taken and the appliances were left in their mouths without any further injection of KT or saline for a period of two weeks.

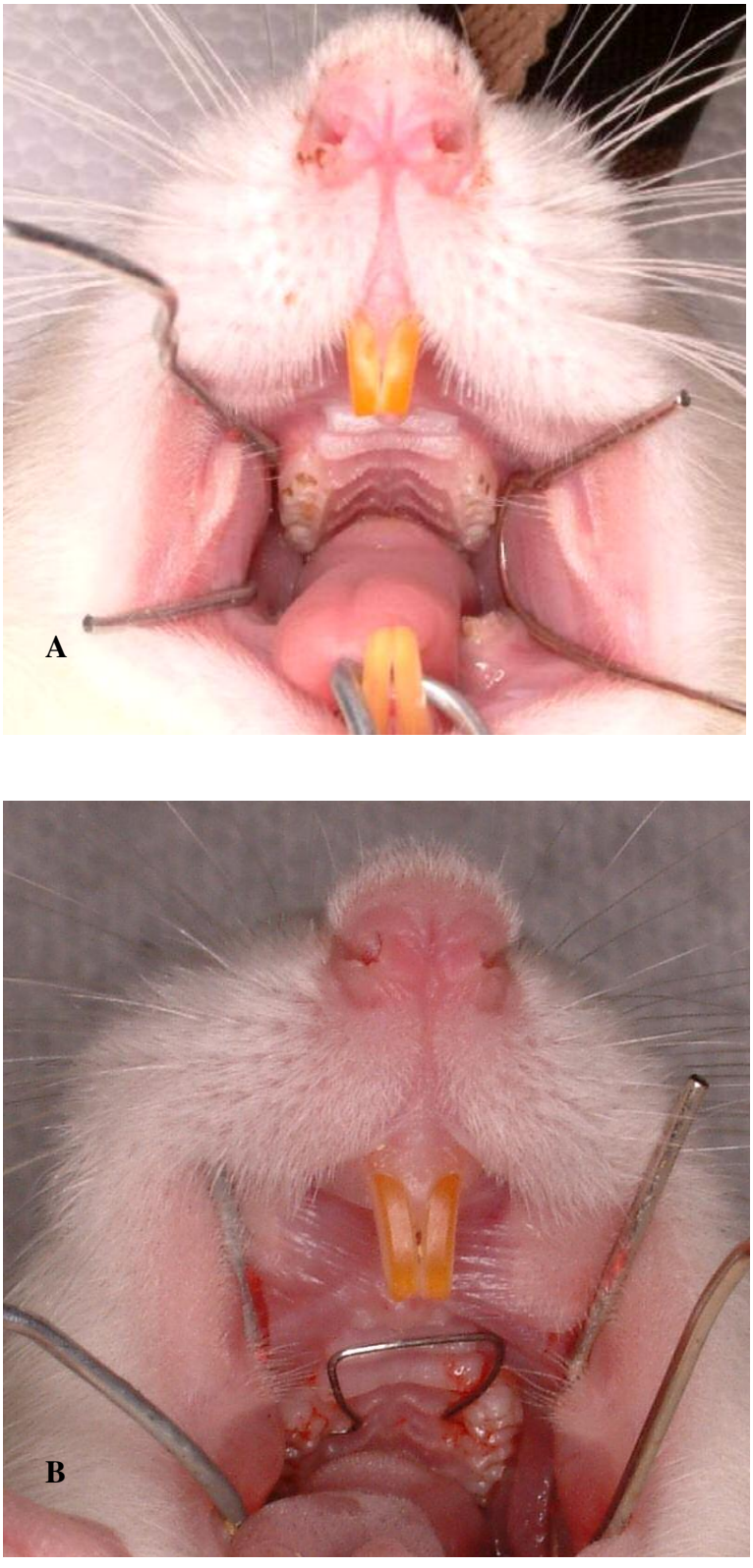

Fig. 7: Rat's mouth before (*A*) and after (*B*) orthodontic spring appliance was positioned between the upper first molars.

Work was not done on the rats during this two week wash-out period. Only the appliances were left in their mouths to allow for continued buccal movement of the first molars. This was done to allow for a more predictable period of relapse tooth movement.

The second part of this experiment, the relapse phase, started at the end of the two week non-intervention, wash-out phase. The relapse period lasted 13 days, allowed for four sessions of general anesthesia, four maxillary impressions (days 1, 5, 9, 13) and three injections of KT and saline (days 1, 5 and 9) per rat. On day one of this phase, the springs were removed (Fig. 8), impressions taken and KT administered around the same teeth as previously done.

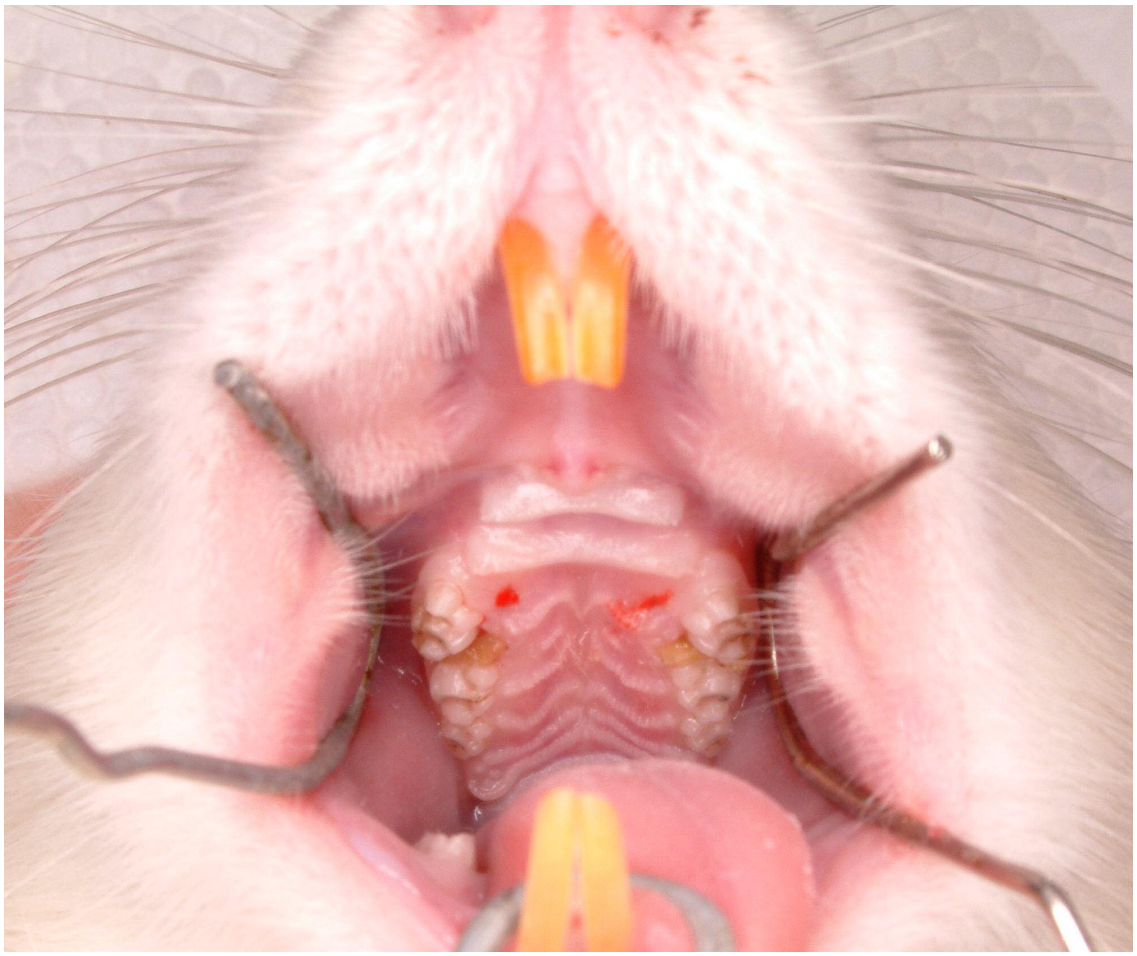

Fig. 8: Day 1 of the relapse phase of the experiment, after the orthodontic appliance was removed from the rat's mouth.

The surgical set-up and procedures were conducted on a Styrofoam pad (Fig. 9). Paper clips, rubber bands, thumb tacks and tape were used to place the rats in the prone position and to help keep their mouths open (Fig. 10). Surgical loops and lights were used often and were available at all times.

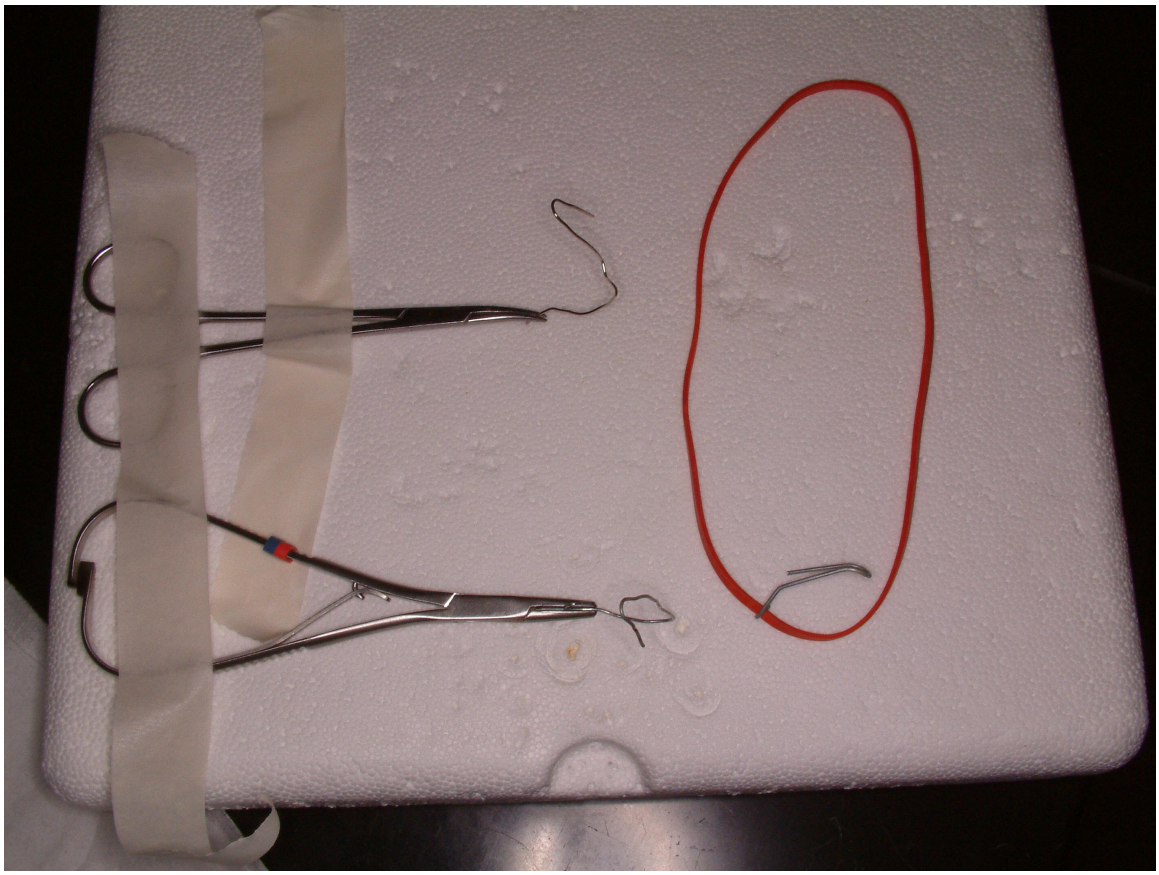

Fig. 9: Styrofoam pad, paper clips, rubber bands and tape used in the surgical set-up.

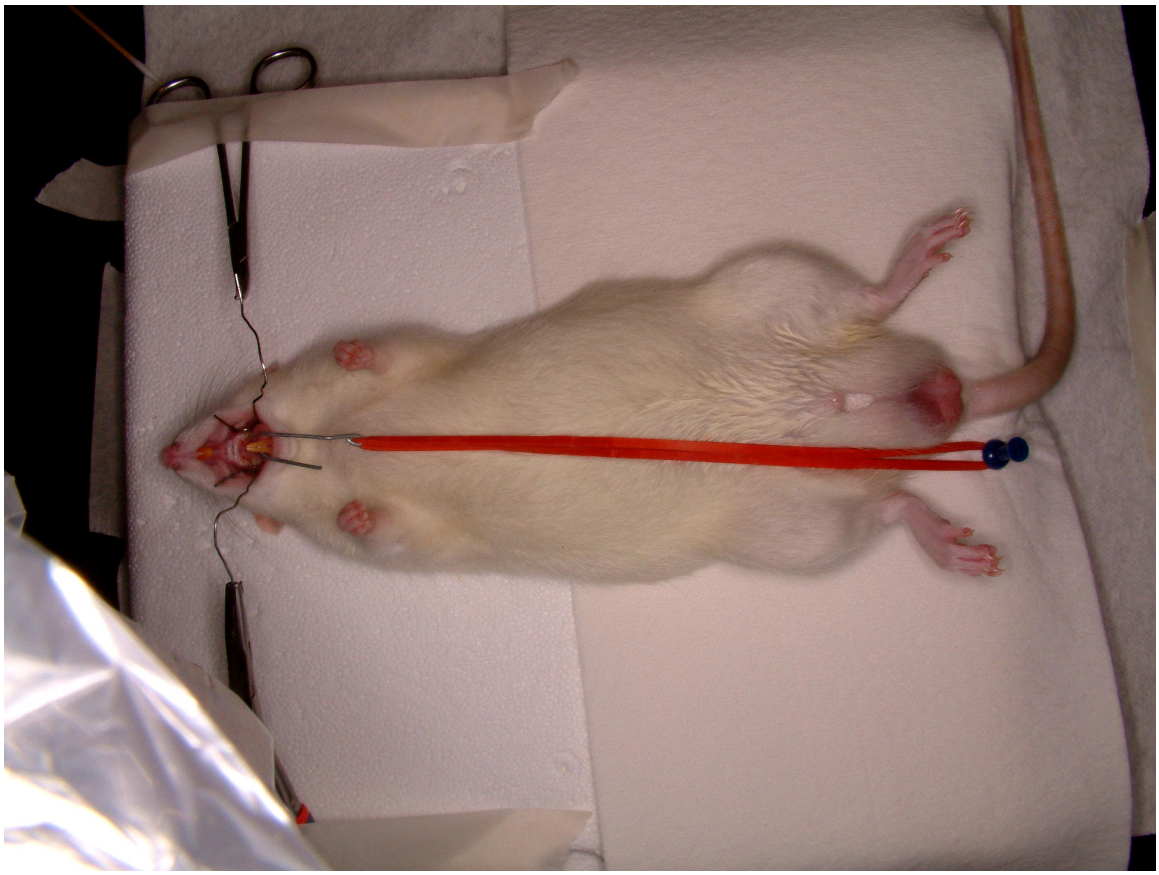

Fig. 10: Position of the rat during the intraoral operative work.

4. Impressions

Wax impressions of the maxillary arch were taken at each time point after the rats were anesthetized and the NSAID and saline were injected. These maxillary impressions were taken using a customized impression tray with red rope wax as the impression material. The impression trays were customized with a handle that allowed an intraoral approach without interference from the upper incisors. Pink Triad® Transheet Visible Light Cure Tray Material, (Dentsply International Inc., York, PA) was used as the base and handle for the

trays. Strips of the soft tray material were cut into pieces measuring 8 mm by 20 mm on a hard acrylic lab cutting board (Fig. 11).

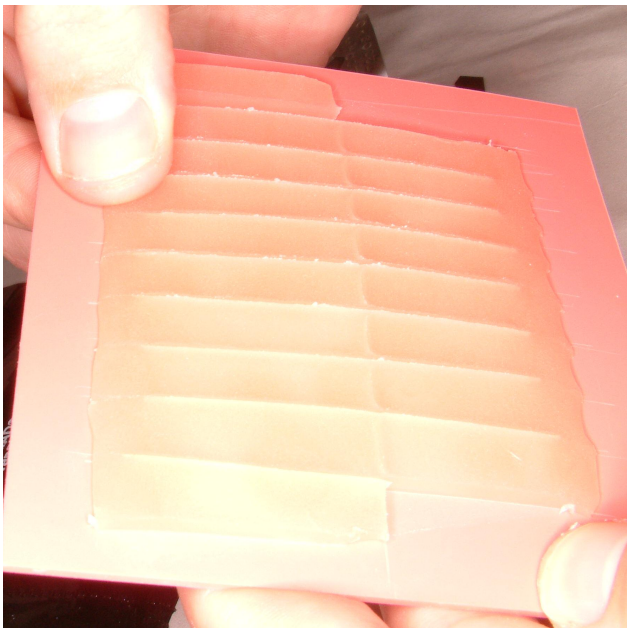

Fig. 11: Rectangular pieces used to make the impression tray bases.

These rectangular pieces of Triad® were light cured in the Dentsply Trubyte Triad® 2000™ (Dentsply International Inc., York, PA) for 4-5 minutes. Oncecured , the pre-cut rectangular pieces of the Triad tray material were trimmed at one end to produce a 45 degree angle to the bottom of the base (Fig. 12).

Fig. 12: Preparation of the 45 degree angle in the tray base.

Fig. 13: Completed impression tray base.

The base (Fig. 13) was then completed. To fabricate the handles of the trays, another sheet of the pink Triad® material was cut into the same dimensions as previously described. Before curing these rectangular pieces, they were first peeled away from the cutting board, hand shaped to resemble an "L". These L shaped rectangular pieces were then adapted to the previously cured flat rectangular tray bases, to be cured and used as handles (Fig. 14).

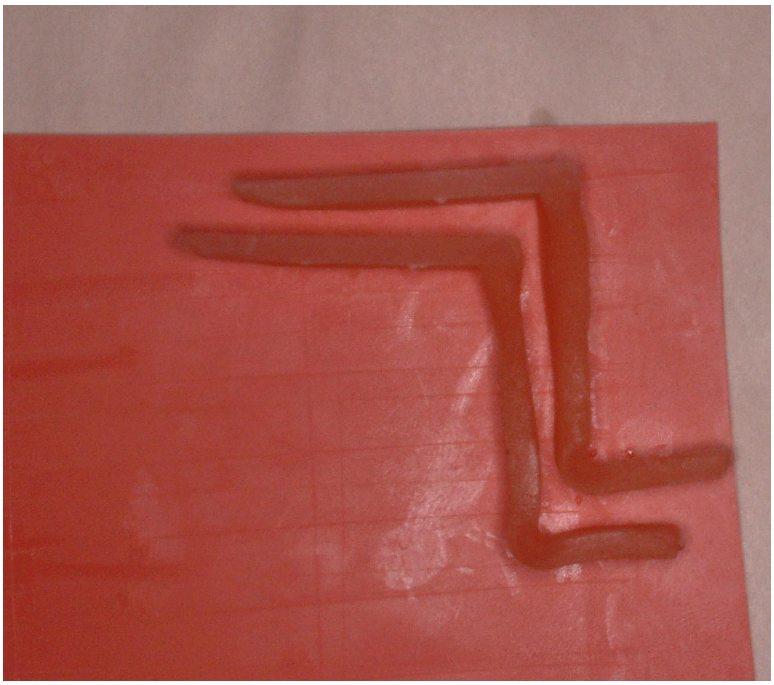

Fig. 14: Preparation of the L shaped handles of the customized impression trays.

For the impression, a strip of red rope wax (Heraeus Kulzer Inc., Armonk, NY) was folded on itself to make it twice as wide. This strip was then cut into pieces that were approximately one cm long (Fig. 15). These rectangular cubes of wax were then adapted to the 45 degree angled base ends and shaped into a flat angulated plane also at the 45 degree incline (Fig. 16).

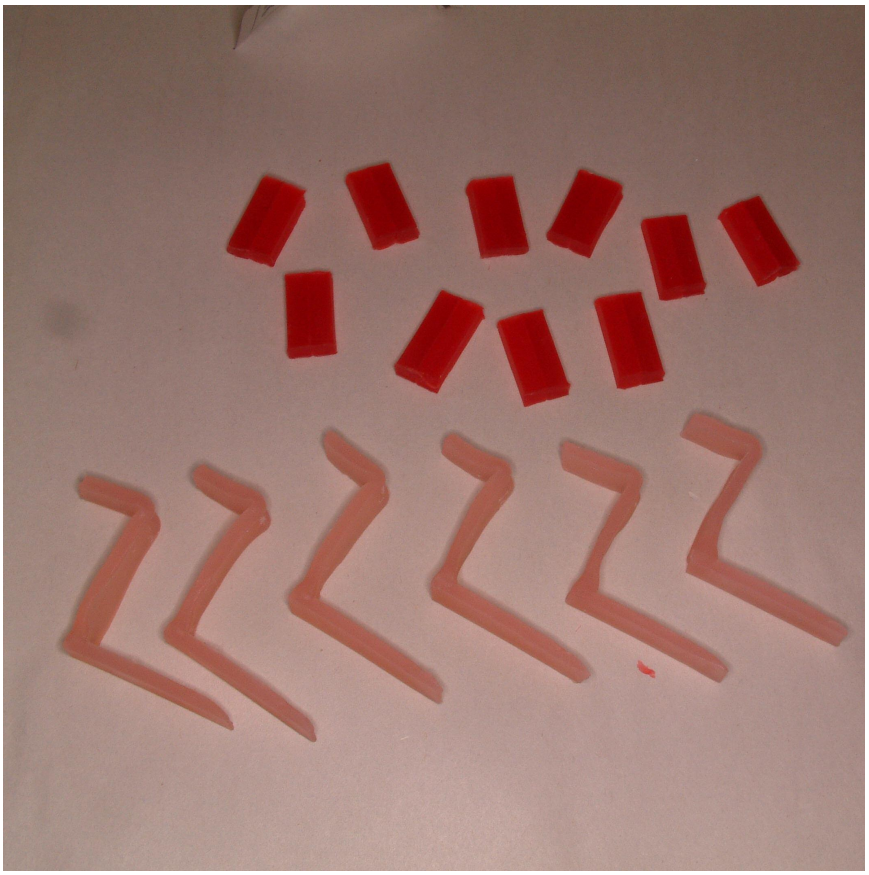

Fig. 15: Rectangular wax cubes ready to be melted onto the custom made impression trays.

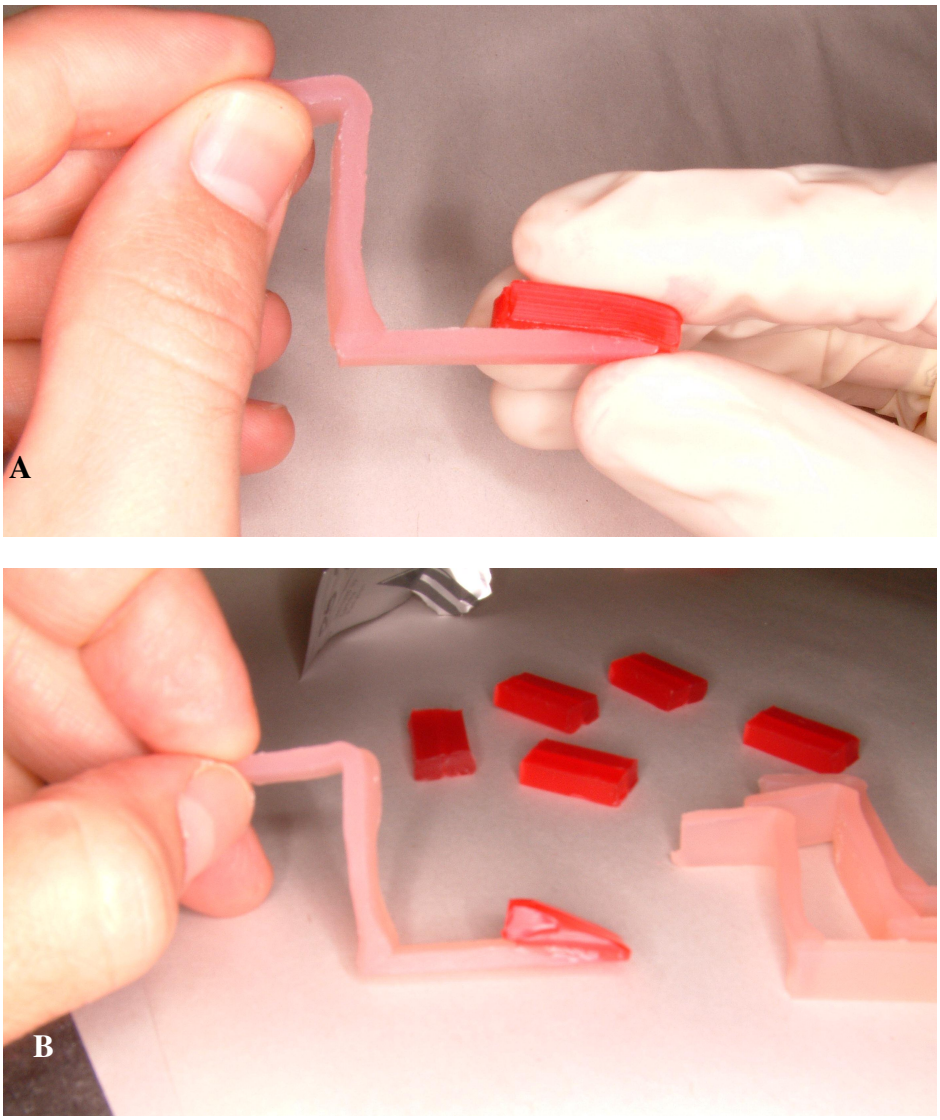

Fig. 16: Wax being adapted onto the impression tray base (*A*) and completed impression tray (*B*).

The bottoms of the trays were left flat which allowed them to be secured properly onto the stage of the scanning electron microscope. An example of an impression is depicted in Fig. 17.

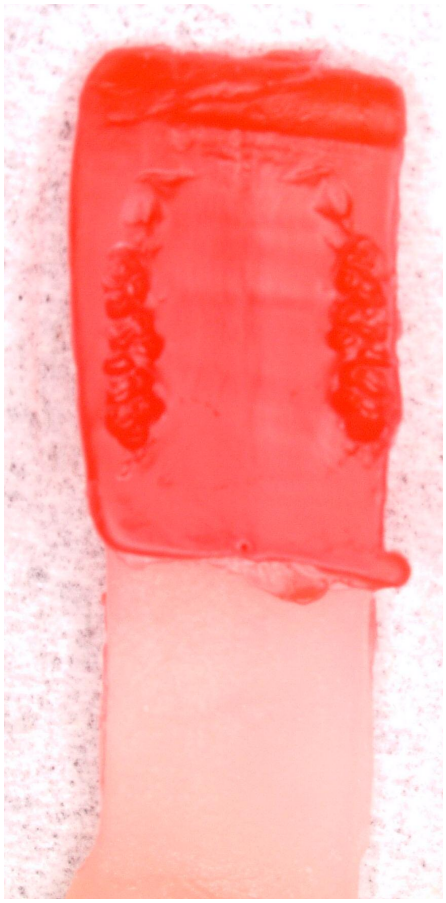

Fig. 17: Example of a rat's maxillary arch impression taken with the custom impression tray.

5. Scanning Electron Microscope Imaging

To archive these impressions, to locate precise reference points and make measurements, these trays went through a standardized protocol prior to scanning them using the scanning electron microscope (SEM). First the trays were covered with a thin conductive film (150 Angstroms) of gold/palladium using a Polaron 5200 sputter coater. These trays were then processed for the SEM by drying the samples in air, mounting them onto specimen stubs with double sided carbon adhesive tabs and colloidal silver paint. The gold/palladium covered trays were then very carefully placed into the JEOL 6300 scanning electron

microscope (Jeol of America Peabody, MA) operating at 10kV. Digital images were collected at 10 x magnification and saved onto a Zip Disc® (Iomega, San Diego, CA). From the Zip Disc® the images were then copied to a SanDisk® 1GB Memory Stick (Sunnyvale, CA). The images were then transferred to an IBM T40 ThinkPad laptop computer (Armonk, New York).

6. Measurement Technique

Tooth movements in each rat were evaluated by measuring the distances the first molars moved relative to a fixed point centered between the upper third molars. To accomplish this, an identifiable point was located on either first molar at each time point for each rat. Another identifiable point was then located on either third molar at each time point. These four points from each impression were overlapped at the center of the line connecting the third molar spots, the reference point (RP). Superimposing the four dots of successive images at the RP allowed the change in position of the first molars at each time point to be measured.

An imaging software program, Image J, (National Institutes of Health, Bethesda, MD) was used in this project for making measurements. It is considered a part of the public domain and is readily available via the Internet (http://rsb.info.nih.gov/ij/). It was used to identify the four position points in each successive image of each rat. The calibrated coordinate system offered in Image J was also utilized. The (X, Y) coordinates for each of these 4 points, were used to translate, rotate and superimpose the images at the RP.

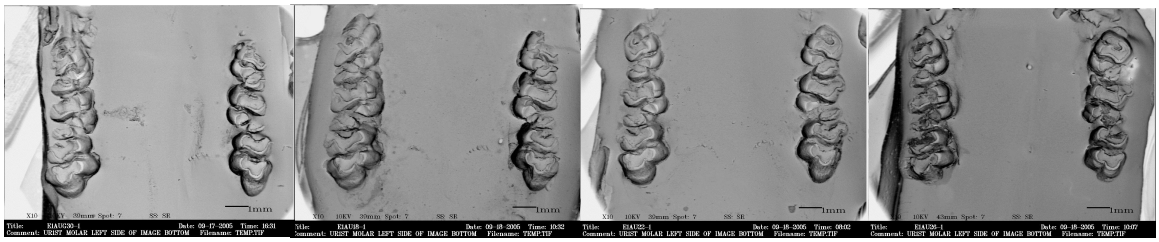

Fig. 18: Successive scanning electron microscope images of rat E1. The images were lined up adjacent to one another and Image J program was used to locate and match the identical 4 points in each image.

Each rat had a series of impressions taken during the expansive and relapse phases of the study. These impressions, after being scanned and saved to a laptop computer, were opened using Image J software. All the successive images were aligned one next to another (Fig. 18). Since each tooth, in each rat, had unique and distinct marks, identifying the same points in each successive impression was possible. We were able therefore to identify and label the same four points in each image. The four points were then numbered conventionally to represent the following. Point 1 (Pt. 1) was always the left posterior identifying point on the upper left (UL) third molar. Pt. 2 was always located on the UL first molar. Pt. 3 was always located on the UR first molar and Pt. 4 was always the right posterior identifying point on the third molar. The center of the line connecting points 1 and 4 (Line 1-4) was the RP where the impression images were superimposed (Fig. 19).

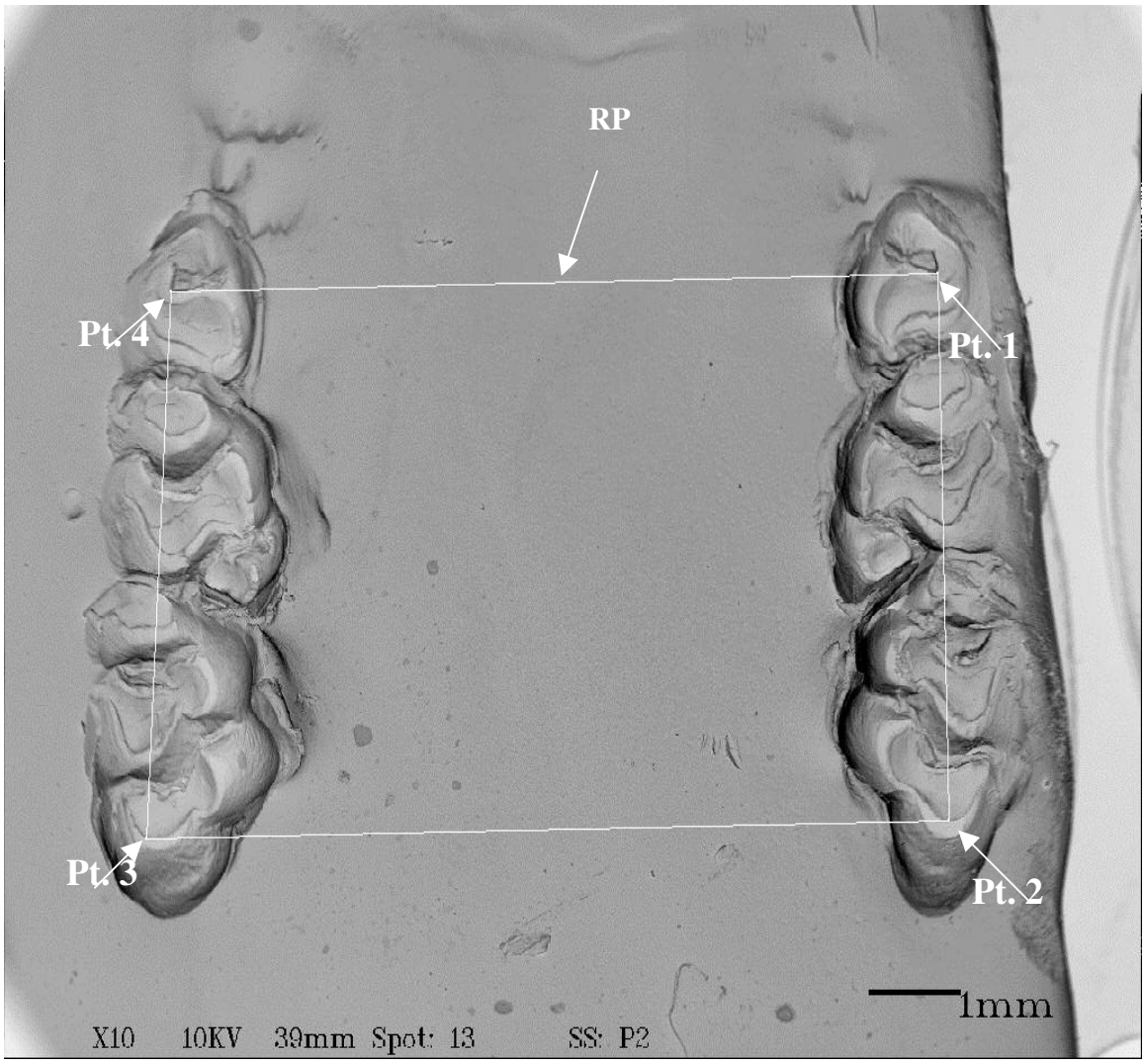

Fig. 19: Scanning electron microscope image of a wax impression. Pts. 1, 2, 3, 4 and RP are indicated by white arrows. Pt. 1 is located on the UL third molar, Pt. 2 is located on the UL first molar, Pt. 3 is located on the UR first molar, and Pt. 4 is located on the UR third molar. Reference point (RP) is the center of Line 1-4. 10x magnification.

Image J, its (X Y) coordinate system and mathematical formulas were used to overlap the points 1 through 4 at the RP. Each set of four points was imagined as being on a two dimensional plane. The planes were then rotated and translated using rotational and translational calculations so that the lines connecting the points 1 and 4 of each image overlapped the original image's Line 1-4. The planes were rotated and translated not only to

overlap one another along Line 1-4, but more precisely, at the midpoints of the lines connecting pts. 1 and 4. Microsoft Excel (Redmond, WA) was used to calculate the rotational and translational figures. Once the images were superimposed at the RP, the distances that the first molars moved relative to where they were at day one, were calculated using the Pythagorean theorem, $a^2 + b^2 = c^2$ where a was the distance moved in the X or horizontal axis, b was the distance moved in the Y or vertical axis and c was the total distance the first molars moved since day 1.

7. Statistical Analysis

The distances measured and reported in the results section are the means, unless otherwise stated, of all the data gathered from all the rats through each phase of the experiment, excluding data from the pilot study. Statistical analyses were conducted using the exact Wilcoxon matched pairs signed rank test, to determine the statisticaldifferences between the measured upper first molar movements for each time point. Differences between movements of the NSAID and saline injected molars were considered significant at $p<0.05$.

III. RESULTS

A. CONSIDERATIONS OF METHODOLOGY

All the rats in this study had normal and consistent weight gain (Appendix A), except two (H1, H2) that lost weight between the wash-out period and the sixth day of the relapse phase due to a malfunction of the water feeding line. Once this problem was corrected, both rats regained normal weight.

There were no appreciable systemic side-effects in any of the rats, such as loss of appetite or change in body weight, and there were no other signs of inflammation such as swelling, redness or bleeding. However, on the fifth day of the relapse phase, rats E1, G1, H1, H2 and on the ninth day of the relapse phase, rats C1, D2 had denuded palates adjacent to the upper first molars where KT was administered. The underlying bone and portion of the palatal root surface of the upper first molars were visible. Tissueinjury was not seen at the site of the saline injections.

During the study, 0.010" SS appliances were retained less predictably than in the pilot study. Three animals had their intraoral expansion springs stay in throughout the experimental period exerting the directional forces desired, but in the remaining nine, the appliances were not always retained in the mouths. In those cases, the springs made of 0.010" SS were replaced with 0.012" SS springs with the same design. Although retention improved with the newer wires, these springs were not always retained in the rat mouths.

B. EXPANSION PHASE

Three out of twelve rats (C1, C2, G2) retained the initial springs at the site of placement throughout the period of investigation. Two rats (D1, E2) did not retain their springs during any of the operative sessions. Three rats (F2, H1, H2) had their springs missing on one of the four days of data collection. One rat (F1) had its appliance missing on two of the four days of data collection and three rats (D2, E1, G1) had their springs missing on three of the data collection days (Table 1).

Four rats (D1, E2, F1, F2) did not have their intraoral springs at the beginning of the relapse phase. Therefore, on day one of what would have been the relapse phase, another expansion spring was positioned transpalatally from upper first molar to upper first molar and the animals went through a second stage of expansion. Three of these four rats (D1, E2, F1) retained their springs at each operative session thereafter while one rat (F2) had the appliance missing at one session. This second set of expansion data for rats D1, E2, F1 and F2 is included in the expansion phase table (Table 1)

EXPANSION PHASE										
			Day 5		Day 9		Day 13		Day 17	
RATS		Site where Ketorolac Tromethamine Delivered: Day 1	Appliance in	Relative movement of KT vs. saline treated teeth (%)	Appliance in	Relative movement of KT vs. saline treated teeth (%)	Appliance in	Relative movement of KT vs. saline treated teeth (%)	Appliance in	Relative movement of KT vs. saline treated teeth (%)
	C1	UR First Molar	Yes	+8.5	Yes	+8.2	Yes	-15.9	Yes:Day 16	+55.6
	C2	UL First Molar	Yes	-6.2	Yes	-8.8	Yes	+6.5	Yes: Day 16	-45.6
	D1	UR First Molar	No: Day 7		No: Day 11		No: Day 15		No: Day 18	
	D2	UL First Molar	No: Day 7		No: Day 11		No: Day 15		Yes: Day 18	-4.4
	E1	UR First Molar	No		No		Yes: Day 14	-1.6	Could not anesthetize	
	E2	UL First Molar	No		Could not anesthetize		No		No	
	F1	UR First Molar	No		Yes	-30.5	Yes: Day 14	+20.9	No	
	F2	UL First Molar	Yes	-28.0	Yes	+7.4	No		Yes: Day 18	-15.6
	G1	UR First Molar	No		No		No		Yes	+23.7
	G2	UL First Molar	Yes	-42.3	Yes	+6.4	Yes	+21.9	Yes	+12.7
	H1	UR First Molar	No		Yes	+30.6	Yes	+14.9	Yes	+67.0
	H2	UL First Molar	No		Yes	-13.1	Yes	-25.9	Yes	+27.1
	D1*	UR First Molar	Yes	+64.8	Yes	+47.3	Yes	-13		
	E2*	UL First Molar	Yes	-47.9	Yes	-32.1	Yes	+19.4		
	F1*	UR First Molar	Yes	-46.2	Yes	-43.2	Yes	-67.5		
	F2*	UL First Molar	No		Yes	-21.9	Yes	-45.3		

Table 1: Appliance retention and relative tooth movement during the expansion phase. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth. *Rats D1, E2, F1, F2 went through a second expansion phase.

KT treated first molars versus saline treated first molars. The calculated distances the upper first molars moved, regardless which side received KT or saline injections, was nearly the same at each time point during the expansion phase. Movement data from each time point of the expansion phase was combined and it revealed that on day 5, the KT treated upper first molars moved 362 μm while the saline treated upper first molars moved 428 μm. On day 9, the KT treated upper first molars moved 444 μm while the control upper first molars moved 465 μm. On day 13, the KT treated upper first molars moved 511 μm and the control upper first molars moved 549 μm. On day 17, the KT treated upper first molars moved 724 μm and the control upper first molars moved 682 μm. Figure 20 graphically displays the mean ± SD distances moved by the KT and saline treated upper first molars at each time point. Differences in movements between the KT treated and saline treated teeth were statistically insignificant at each time point. The individual data of each rat during the expansion phase are reported in the appendices.

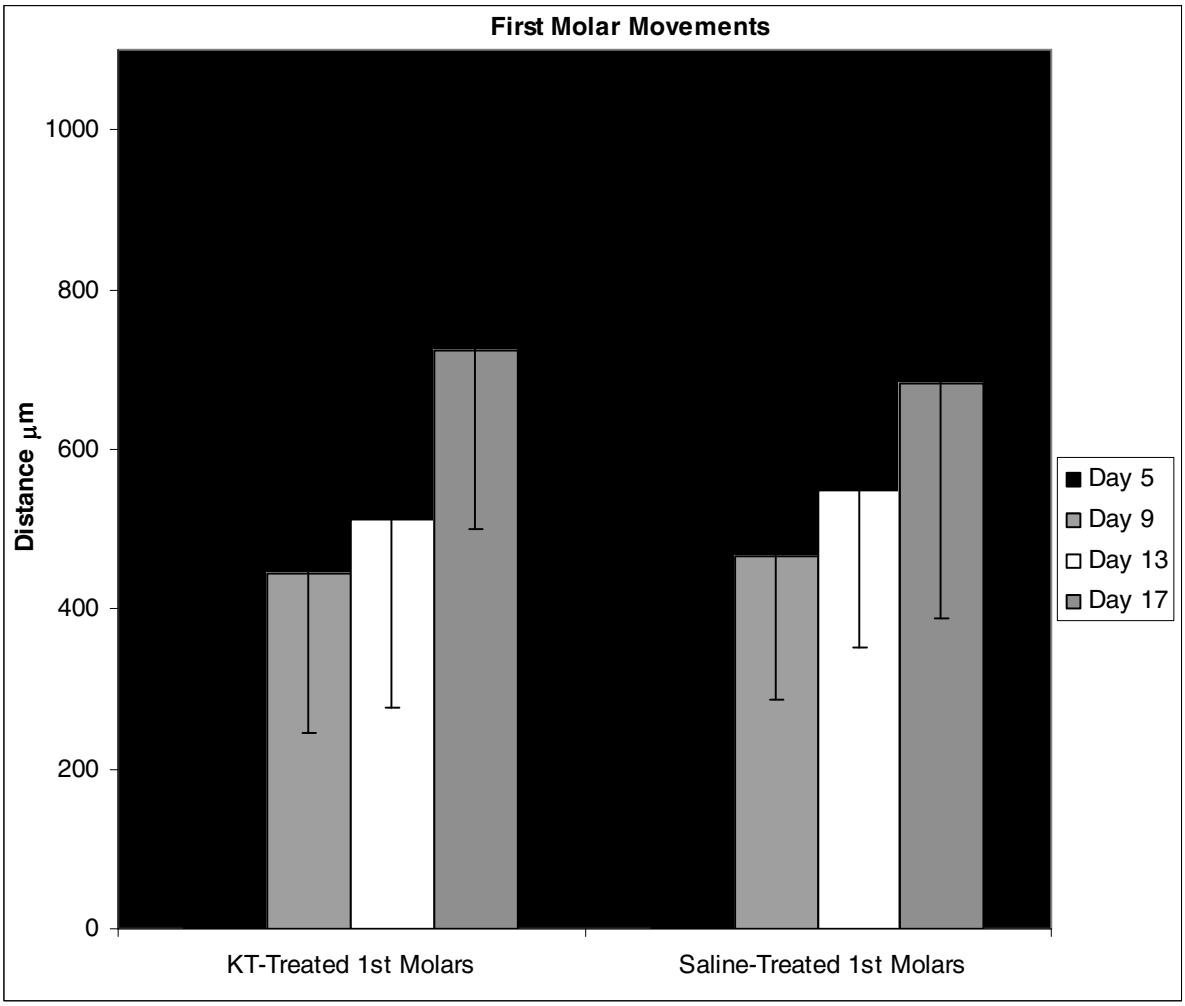

Figure 20: Time-course movements of KT and saline treated first molars during the expansion phase. Each column represents the mean distance (µm) ± SD. The sample sizes were: *n*=7 on day 5, *n*=11 on day 9, *n*=11 on day 13, *n*=8 on day 17.

C. RELAPSE PHASE

Eight rats (C1, C2, D2, E1, G1, G2, H1, H2) went through the relapse phase of this study (Table 2). On day one, the expansion springs that were retained during the two week wash-out period were removed. Four rats (G1, G2, H1, H2) had their impressions taken on day one of the relapse phase just before the springs were removed, and four rats (C1, C2, D2, E1) had their impressions taken just after the springs were removed. Four of the animals (C1, E1, G1, H1) had the KT delivered locally adjacent to the UR first molars and the other four rats (C2, D2, G2, H2) had it delivered around the UL first molars. Six of the eight rats (C1, C2, D2, E1, G1, G2) had KT injected and impressions taken on days 1, 5, 9 and 13. Two of the rats (H1, H2) had KT injected and impressions taken on days 1, 6, 10 and 13.

RELAPSE PHASE										
			Day 1		Day 5		Day 9		Day 13	
RATS		Site where Ketorolac Tromethamine Delivered: Day 1	Appliance in		Appliance in	Relative movement of KT vs. saline treated teeth (%)	Appliance in	Relative movement of KT vs. saline treated teeth (%)	Appliance in	Relative movement of KT vs. saline treated teeth (%)
	C1	UR First Molar	Yes			+1.3		+311.1		+339.2
	C2	UL First Molar	Yes			-56.5		-73.1		-10.3
	D1**	UR First Molar	No	New Appliance						
	D2	UL First Molar	Yes			-93.9		-5.4		+143.3
	E1	UR First Molar	Yes			+20.7		-9.1		+7.1
	E2**	UL First Molar	No	New Appliance						
	F1**	UR First Molar	No	New Appliance						
	F2**	UL First Molar	No	New Appliance						
	G1	UR First Molar	Yes			+35.5		+192.1		-1.1
	G2	UL First Molar	Yes			-63.7		+106.2		-2.8
	H1	UR First Molar	Yes		Day 6	+4.2	Day 10	same		+39.4
	H2	UL First Molar	Yes		Day 6	+203.4	Day 10	+43.1	Day 14	+8.1

Table 2: Appliance retention and relative tooth movement during the relapse phase. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth. ** Rats D1, E2, F1 and F2 did not have relapse data because on day one of what should have been the relapse phase, their springs were missing. They underwent a second expansion phase and their data are listed with the expansion phase data.

KT treated first molars versus saline treated first molars. The calculated distances the upper first molars moved, regardless which side received KT or saline injections, was nearly the same at each time point during the relapse phase. Movement data from each time point during the relapse phase were combined and it revealed that on day 5, KT treated upper first molars moved 147 μm and the saline treated upper first molars moved 170 μm. On day 9, KT treated upper first molars moved 239 μm while the saline treated upper first molars moved 183 μm. On day 13, the KT treated upper first molars moved 222 μm and the control upper first molars moved 177 μm. Figure 21 graphically displays the mean ± SD distances moved by the KT and saline treated upper first molars at each time point The differences in the calculated movements were not statistically significant. The individual data of each rat during the relapse phase are reported in the appendices.

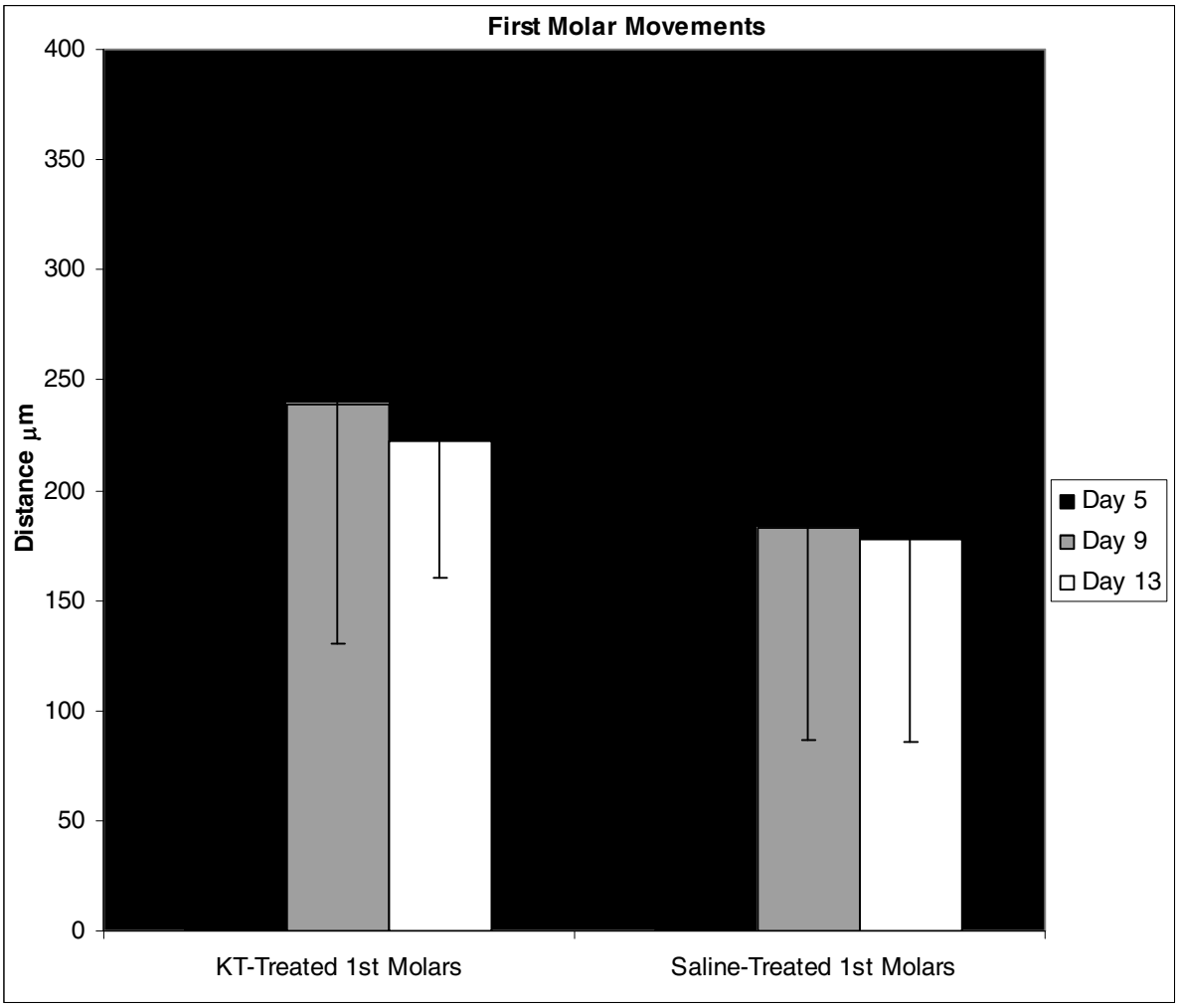

Figure 21: Time-course movements of KT and saline treated first molars during the relapse phase. Each column represents the mean distance (µm) ± SD. The sample sizes were: *n*=8 on day 5, *n*=8 on day 9 and *n*=8 on day 13.

IV. DISCUSSION

It is well documented that teeth translate through bone by means of a remodeling process promoted by the inflammatory response (Proffit, 2000). Inflammation results from a cascade of biological events and PGs have been identified as key mediators for osteoclastic activity leading to bone resorption (Klein and Raisz, 1970). Investigators used these findings to effect tooth movement rates by altering PGE_2 levels in their experimental subjects. Yamasaki *et al.,* (1982, 1984) injected exogenous PGE_1 and PGE_2 locally in monkeys and humans and increased the rates of tooth movement. Chumbley and Tuncay, (1986) and Zhou *et al.,* (1997) used systemically administered PGE_2 inhibitors to decrease the overall tooth movement rates in cats and rats.

In our study, we attempted to effect tooth movement rates by delivering a potent anti-inflammatory drug adjacent to teeth hoping to increase their anchorage value. Our hypothesis was that, by delivering a NSAID locally, the PGE_2 production at that site would diminish. This would decrease the bone resorption rate at the site, which in turn would cause a decrease in tooth movement. The results from our investigation revealed thatthe movements of the upper first molars, during the expansion and relapse phases, were similar regardless if they received ketorolac tromethamine or saline. The differences in tooth movements were not statistically significant. To help explain these results, the methods used in this study may need to be examined in detail.

A. CONSIDERATIONS OF METHODOLOGY

1. ANIMAL

The rat model has been used in past toxicological and orthodontic tooth movement studies (Yamasaki *et al.,* 1982,1984; Mohammed *et al*, 1989, Vandevska -Radunovic *et al*, 1994; Leiker *et al.*, 1995; Boekenoogen *et al*, 1996; Soma *et al*, 1999; Kyrkanides *et al*, 2000; Kalia *et al*, 2003; Dolce *et al.*, 2003). Jee *et al.*, (1991), and Ren, *et al.*, (2004), report that the rat animal model is suitable for such investigations because of their skeletal adaptation to mechanical forces. Although the rat oral cavity and dentition were small, it was relatively easy to insert a fixed orthodontic appliance and take intraoral maxillary impressions. However, we encountered some technical difficulties in administering submucosal injections into the rat palate around the investigative teeth. Due to the thin and fibrous rat palatal tissues, occasionally we observed some leakage during the placement of the saline or KT solutions, suggesting that the desired KT concentration may not have been consistently achieved in all the experimental periods. We also noted mucosal stripping of palatal tissues in several rats at the site of KT injections during the relapse phase. The volume of the drug or the concentration administered beneath the thin palatal mucosa could have contributed to this effect, masking the anti-inflammatory action of the drug.

2. PHARMACEUTICAL AGENT

Our choice to test ketorolac tromethamine as our investigative NSAID was dictated by three reasons; its anti-inflammatory potency (Allison *et al.,* 1993), effectiveness in its topical application in inhibiting PGE_2 production (Kelm *et al*, 1996) and its tissue tolerance upon local injection (Chellman *et al.,* 1994). Kelm *et al.,* (1996) showed that KT

significantly reduced PGE_2 levels in the GCF when it was locally administered, two times daily, for eight days in humans. They also showed that the half life of KT within the GCF was 30 minutes. The schedule used during our study for delivering KT was once every four days. It is possible the frequency of the drug delivery was not often enough to clinically inhibit tooth movement.

KT was found to be well tolerated when it was injected into the paws and muscles of rats (Chellman *et al.,* 1994) and non-irritatingwhen applied to human mucosal tissues (Kelm *et al.,* 1996; Jeffcoat *et al.,* 1995). However the KT solution used in our study also contained ethanol, 10% w/v A severe synovial inflammation was found in rat knees after five days following a single KT injection to that site, but not in the control knees injected with a saline solution (Irwin *et al.,* 1998). The authors investigated whether the ethanol could have been responsible for the inflammation, but they found the application of 10% w/v ethanol by itself did not elicit any inflammatory response in the rat synovial tissue. Based on these observations, even though the anatomical and histological characteristics of the rat synovia may not be exactly comparable to those of the palatal mucosa, we could speculate that the inflammatory reaction could have been caused by the three-week long exposure to localized KT. However, we could not exclude ethanol either directly causing or contributing to the necrosis observed in some of the rat palatal tissues. Injections of a 10% w/v ethanol solution would help clarify this point. At this point, it is unclear whether the drug concentration, volume, alcohol content or the method of application contributed to the stripping of the palatal tissues.

It has been reported that indomethacin not only inhibits PG synthesis but is also a potent inhibitor of cAMP-dependent protein kinase (Kantor and Hampton, 1978). These

protein kinases may in turn have a significant influence on the activity of osteoclasts. Thus indomethacin may have affected tooth movement in previous studiesnot only by inhibiting PG production, but probably by interfering with other biologic processes too. KT is a potent PG inhibitor however it is known that PGs are not the only mediators of bone resorption associated with tooth movement. A more ideal NSAID could be used in future studies.

3. ORTHODONTIC APPLIANCE

In the design of our experimental protocol and orthodontic appliance, we tried to replicate the work of Igarashi *et al.,* (1994). In their studies, they placed intraoral springs made of 0.012" NiTi wire and were able to demonstrate good retention of the springs. However, our attempts to achieve consistent retention of that type of appliance in the rat mouths were not successful. In our hands, the 0.012" NiTi wires that we initially used in the pilot study were not retained by the rats. To improve the retention of the springs, the composition and stiffness of the wires were changed to 0.010" SS, maintaining the appliance design. However, their retention remained inconsistent during the actual study. The springs were retained in the rat mouths more predictably as we further increased the stiffness of the wires, passing to a 0.012" SS wire. These springs increased the force levels applied to the rats' teeth from 10.3 g produced by the NiTi springs to 59.3 g, at a deflection of 1.5 mm. It is possible this large increase in applied force levels may have exceeded the pharmaceutical effect or capability of the locally delivered KT. Wong, *et al*, (1992) suggested that forces of 25 g or more might stimulate maximal bone resorption and tooth movement overwhelming any inhibitory effects of an anti-inflammatory drug in the guinea pig. Since rats have similar to smaller sized teeth, the forces used in our study may have been too high, overpowering the anti-inflammatory effects of KT.

4. MEASUREMENT TECHNIQUE

Our measurement technique involved the identification of four arbitrary points, each one located on a different tooth. Of the four points identified from each impression, the two localized on the third molars, were chosen as fiduciary points and they were used to help superimpose the images for calculating differential tooth movements of both upper first molars. Ideally, fiduciary points should be completely stationary at all times to allow absolute measurements. Due to the growth of the young rats, whose weights were almost doubled by the end of the experiment, from the consecutive sets of images, we observed that, in addition to the first molars, the two fiduciary points on the third molars also moved, even though slightly, during the expansion and relapse phases. Therefore, the tooth movements were measured from a single reference point that was the midpoint of the line that connected the two fiduciary points. Since these two fiduciary points moved throughout the study, the point where all the images were superimposed, the RP was not truly fixed. Therefore the measurements of the differential first molar movements may not be accurate.

B. INTERPRETATION OF RESULTS

Pharmaceutical manipulation of the inflammatory response during orthodontics to increase and decrease tooth movement rates has been demonstrated. Systemically administered PG inhibitors can cause tooth movement rates to decrease. Indomethacin and ibuprofen, two different PG inhibitors, were used by Zhou *et al.,* (1997), Kehoe *et al.,* (1996), Mohammed *et al.,* (1989), Chumbley and Tuncay (1986) to decrease tooth movement in their animal models. A bisphosphonate drug, another type of osteoclast-mediated bone

resorption inhibitor, normally prescribed to counter osteoporosis, proved to decrease tooth movement rates in rats when systemically and locally administered (Igarashi *et al.,* 1994).

Our hypothesis of applying NSAIDs locally to take advantage of their anti-inflammatory effects, at the scene of application, to increase the anchorage value of specific teeth, remains a theory since this study did notprove our hypothesis to be true. However, by modifying some of the methods used, it is possible that favorable outcomes may be achieved.

C. FUTURE WORK

During this study, injection of the KT solution under the thin palatal tissues posed several problems that should be addressed in the future by changing the drug delivery methodology. The use of subcutaneous, surgically positioned, sustained-release microspheres, from which a pharmacological agent can be delivered at sufficient levels throughout the study period, is an option that might be considered (Sinha *et al.*, 2005; Dolce *et al.,* 2003). Alternatively, it is possible to implant osmotic pumps in the subcutus of the dorsocervical region of rats to release an NSAID near a specific target (Soma *et al.,* 1999). The continuous infusion of KT to localized sites would eliminate three problems encountered in our study: 1) the need for periodical, high volume injections of the NSAID, 2) the problem of leakage from the injection sites and 3) the possible presence of toxic drug solvents such as ethanol. The time sustained drug delivery by microspheres could allow the investigator to reduce the amount of drug used and still observe inhibition in tooth movement. In fact, a lower, but constant, local concentration of the KT, that would otherwise require more frequent injections, would address the issue of the short half-life of KT within the GCF.

Furthermore, this experimental approach might diminish the local side-effects that could have been caused by the high volume or higher concentration of KT at the site of injection.

Future work will also benefit from a redesign of our intraoral appliance. Even though the appliance that we used in our study had the advantages of being simple and easy to position in the rat's mouth, it was often ineffective due to its poor retention. Using a cemented or permanently bonded spring would eliminate a few uncontrollable factors, such as the rat's innate desire and ability to remove an intraoral appliance. In a revised protocol, an appliance that is secured in the rat's mouth could be made of 0.012" NiTi wire. This type of wire, unlike the SS wire of the same diameter, has the intrinsic property of yielding low forces with large deflections. In our experimental conditions, the NiTi spring exerted forces that were within a physiological range (Ren *et al.,* 2004) and more likely to be within the effective pharmaceutical range of KT.

Additionally, in our preliminary study, we attempted to measure the PG levels in the GCF, but we were unable to fit the detection paper strip inside the rat gingival crevice. However, we do believe the measurements of PGs in the GCF and the histological examination of the KT treated maxilla and its soft tissues would be very useful. Histological examinations may help determine the lowest effective KT concentration levels that give the greatest benefit of decreased inflammatory mediators. This approach would also help elucidate whether chronic exposure to KT could cause undesired side effects, such as necrosis and denuding of palatal tissues.

Lastly, a fixed fiduciary point, one that does not move is important when superimposing several images on one another to measure relative tooth movements. Bjork,

(1968) studied the growth rates and patterns in human patients by superimposing several cephalometric images, on the presence of fixed metallic implants. It would be advantageous for future experiments to insert metallic implants on either side of the rat palatal midline, towards the posterior part of the oral cavity, to be used as fixed points, thus reliable, reference points to superimpose the consecutive SEM images.

This research project began with the idea of utilizing bioresorbable, sustained release, microspheres as a vehicle to painlessly deliver a NSAID topically to inhibit tooth movement. Although we were not successful in using this animal model, there were sufficient methodology issues that call for this idea to be studied further. It remains possible, that by modifying the methods used in this study and incorporating this innovative concept of gently placing bioresorbable microspheres directly into the gingival sulci of larger animals and perhaps one day in humans, anchorage values of teeth could be enhanced. If so, this may become an alternative to temporary skeletal anchorage screws and pins.

APPENDIX A
RAT WEIGHT CHANGES DURING THE EXPERIMENTAL STUDY

EXPANSION PHASE

Rat weight (g)

Day		**C1**	**C2**	**D1**	**D2**	**E1**	**E2**	**F1**	**F2**	**G1**	**G2**	**H1**	**H2**
1		210	230	210	210	215	225	321	300	280	280	330	315
5		244	252			251	242	350	335	295	295	335	320
7				216	249								
9		269	266			277	274	385	360	300	305	345	340
11				289	275								
13		308	280						380	315		350	335
14						305	305	395					
15				324	298								
16		290	275										
17							282	415	380	315	315	385	360
18				290	277								

Table A1: Weight of rats during expansion phase.

RELAPSE PHASE

Rat weight (g)

Day		**C1**	**C2**	**D1**	**D2**	**E1**	**E2**	**F1**	**F2**	**G1**	**G2**	**H1**	**H2**
1		430	370	450	415	420	405	505	460	360	365	310	280
5		420	350	450	415	420	410	520	465	370	365		
6												280	250
9		420	345	450	410	425	415	515	465	380	365		
10												290	280
13		425	360	465	430	440	430	525	480	390	360	345	
14													330

Table A2: Weight of rats during relapse phase.

APPENDIX B

SUMMARY OF RAT C1 EXPANSION PHASE DATA

Rat C1 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UR 1st molar (Pt. 3)	Pt. #2	179.10	499.53	530.66	+8.45
		Pt. #3	-547.97	175.93	575.52	
Day 9	UR 1st molar (Pt. 3)	Pt. #2	612.26	267.43	668.12	+8.16
		Pt. #3	-620.34	370.65	722.64	
Day 13	UR 1st molar (Pt. 3)	Pt. #2	901.12	106.48	907.39	-15.90
		Pt. #3	-710.50	278.49	763.13	
Day 16	UR 1st molar (Pt. 3)	Pt. #2	658.52	141.20	673.48	+55.62
		Pt. #3	-1035.12	164.25	1048.07	

Table B1: Rat C1 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat C1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movements of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

Figure B1: Rat C1 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

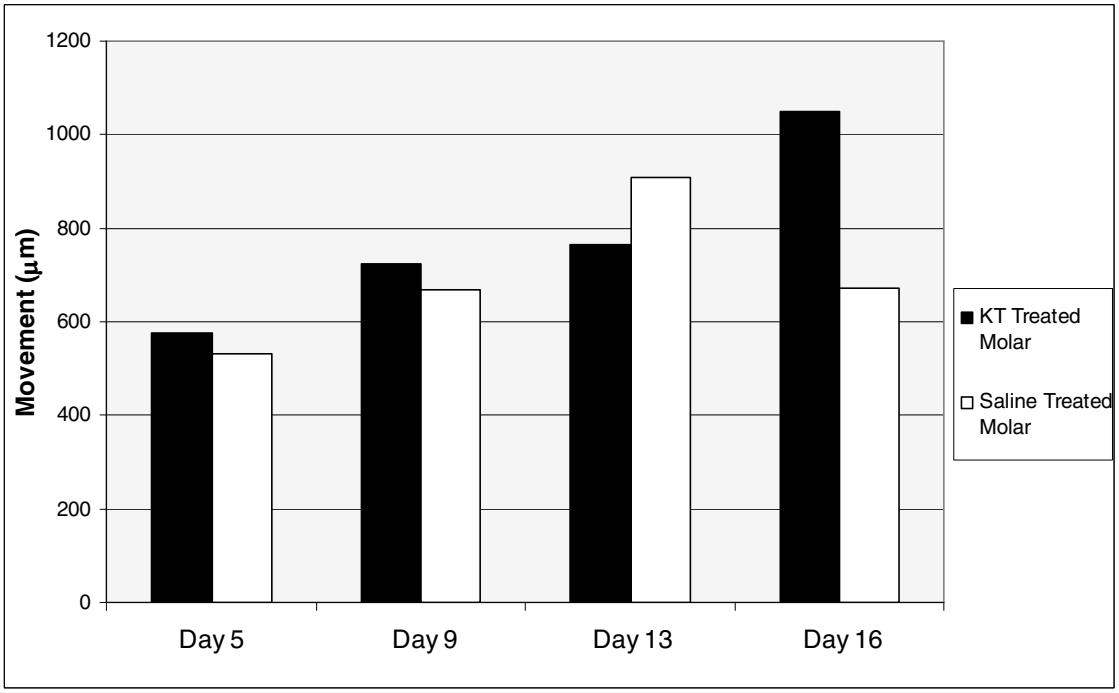

Figure B2: Time-course movements of KT and saline treated first molars during the expansion phase in rat C1. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX C

SUMMARY OF RAT C2 EXPANSION PHASE DATA

Rat C2 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UL 1st molar (Pt. 2)	Pt. #2	360.40	255.34	441.68	-6.19
		Pt. #3	-458.23	108.19	470.83	
Day 9	UL 1st molar (Pt. 2)	Pt. #2	715.14	86.81	720.39	-8.80
		Pt. #3	-769.46	178.53	789.90	
Day 13	UL 1st molar (Pt. 2)	Pt. #2	895.91	-64.89	898.26	+6.07
		Pt. #3	-835.80	115.31	843.71	
Day 16	UL 1st molar (Pt. 2)	Pt. #2	588.31	274.56	649.22	-83.90
		Pt. #3	-1189.89	97.93	1193.91	

Table C1: Rat C2 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat C2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movements of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

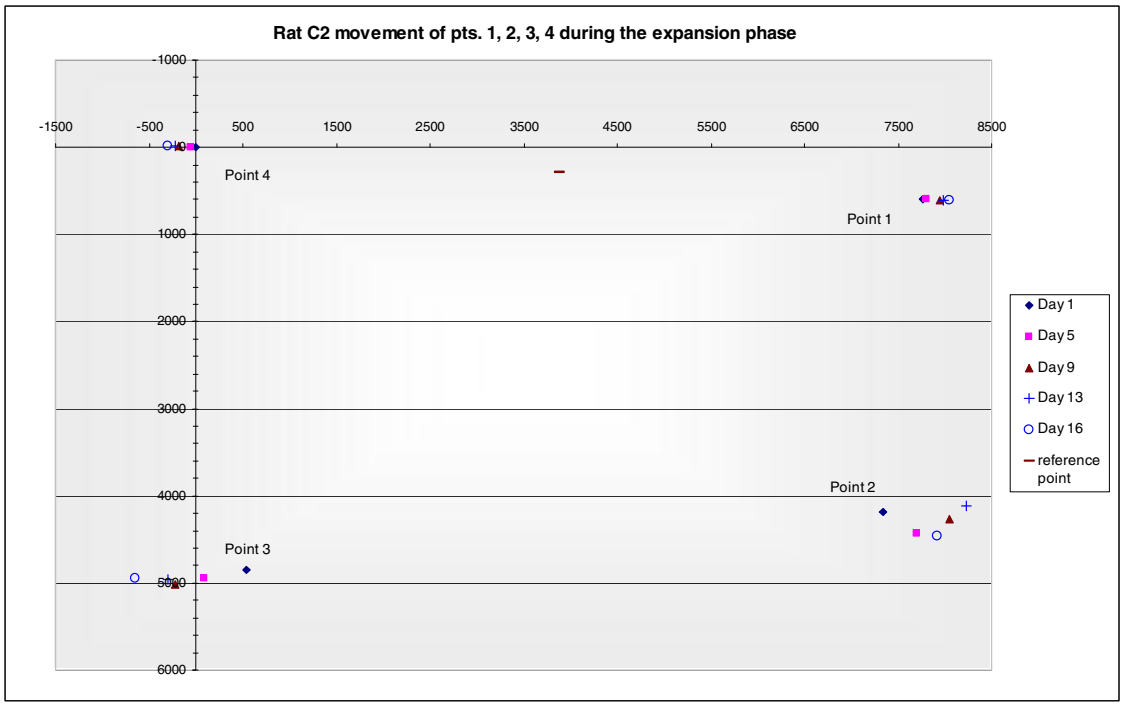

Figure C1: Rat C2 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

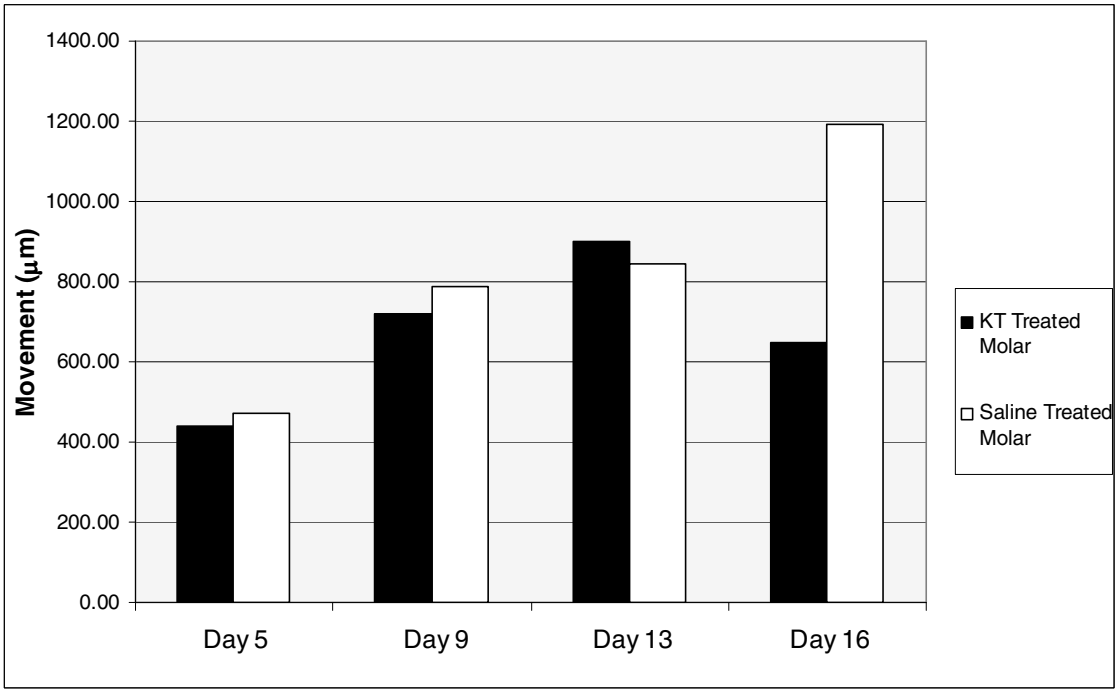

Figure C2: Time-course movements of KT and saline treated first molars during the expansion phase in rat C2. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX D

SUMMARY OF RAT D2 EXPANSION PHASE DATA

Rat D2 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 7	UL 1st molar (Pt. 2)	Pt. #2				
		Pt. #3				
Day 11	UL 1st molar (Pt. 2)	Pt. #2				
		Pt. #3				
Day 15	UL 1st molar (Pt. 2)	Pt. #2				
		Pt. #3				
Day 18	UL 1st molar (Pt. 2)	Pt. #2	306.72	-63.64	313.26	-4.42
		Pt. #3	-318.32	78.10	327.76	

Table D1: Rat D2 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat D2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movement of pts. 2 and 3 was measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movement of the KT treated tooth was calculated relative to the saline treated tooth.

Figure D1: Rat D2 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

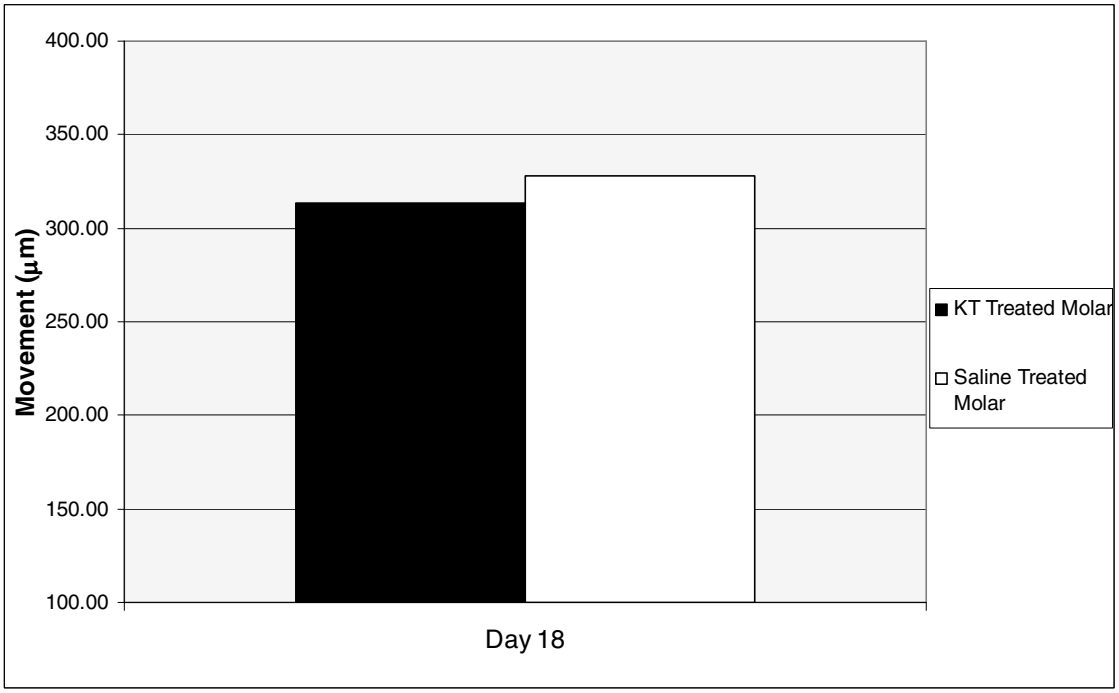

Figure D2: Time-course movements of KT and saline treated first molars during the expansion phase in rat D2. The distance (µm) moved by the KT treated tooth (*black bar*) and by the saline treated tooth (*white bar*) is given at day 18.

APPENDIX E

SUMMARY OF RAT E1 EXPANSION PHASE DATA

Rat E1 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UR 1st molar (Pt. 3)					
Day 9	UR 1st molar (Pt. 3)					
Day 14	UR 1st molar (Pt. 3)	Pt. #2	432.85	347.66	555.18	-1.64
		Pt. #3	-524.69	151.28	546.06	
Day 16	UR 1st molar (Pt. 3)					

Table E1: Rat E1 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat E1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movement of pts. 2 and 3 was measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movement of the KT treated tooth was calculated relative to the saline treated tooth.

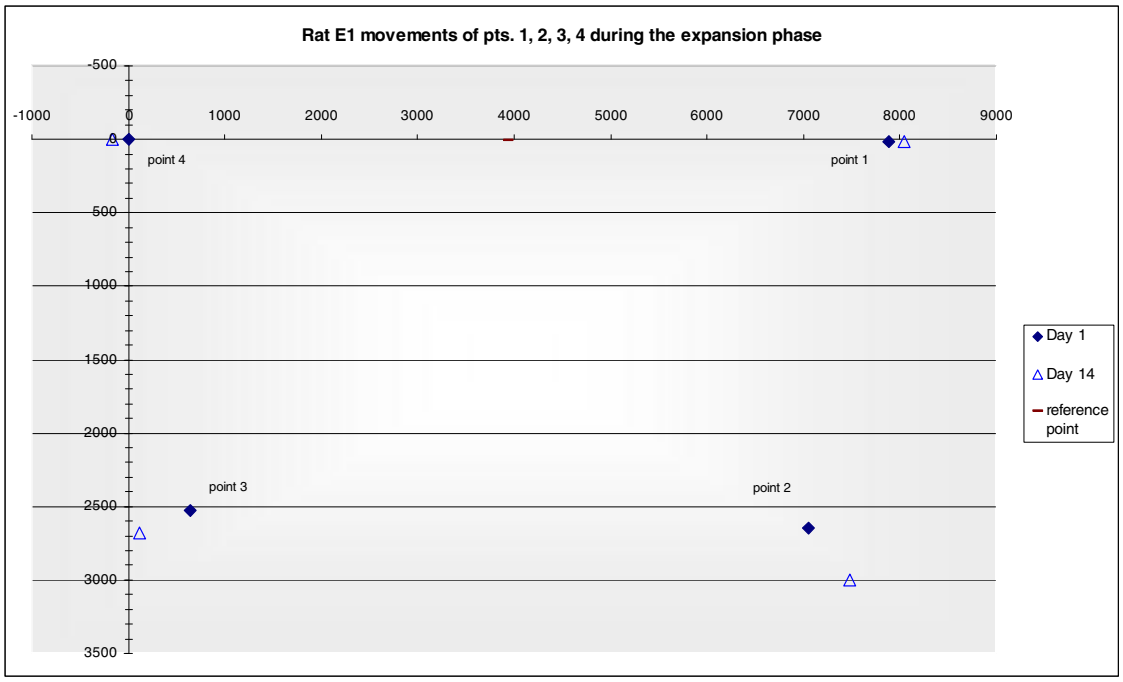

Figure E1: Rat E1 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

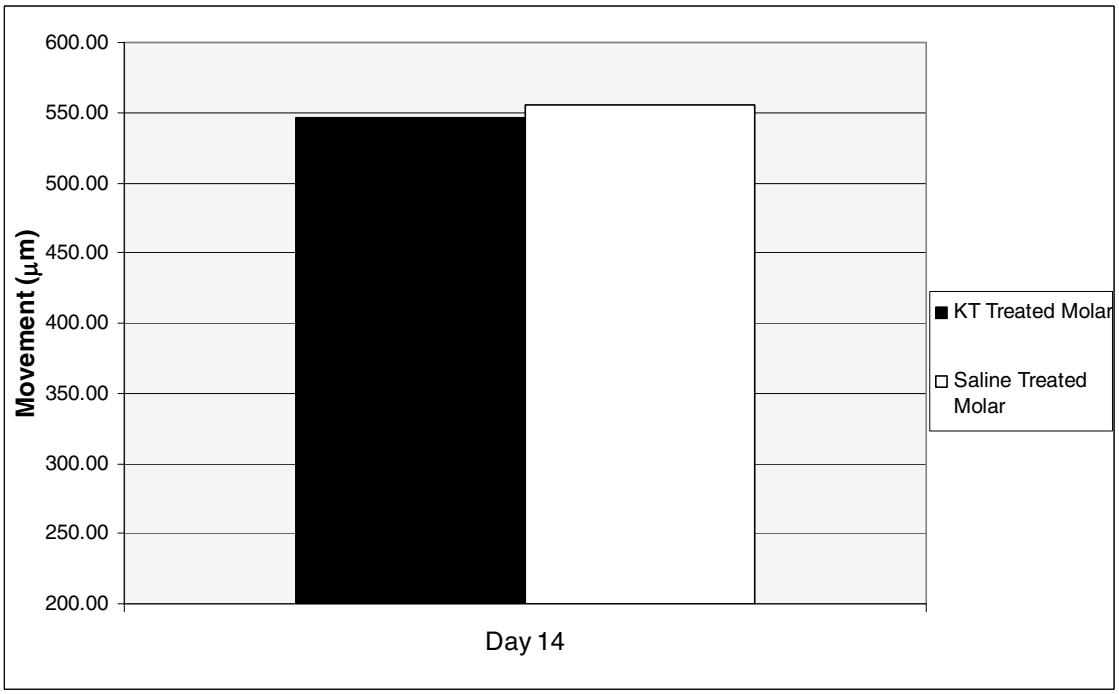

Figure E2: Time-course movements of KT and saline treated first molars during the expansion phase in rat E1. The distance (µm) moved by the KT treated tooth (*black bar*) and by the saline treated tooth (*white bar*) is given at day 14.

APPENDIX F

SUMMARY OF RAT F1 EXPANSION PHASE DATA

Rat F1 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UR 1st molar (Pt. 3)	Pt. #2				
		Pt. #3				
Day 9	UR 1st molar (Pt. 3)	Pt. #2	462.23	-62.84	466.48	-30.51
		Pt. #3	-116.00	302.66	324.13	
Day 14	UR 1st molar (Pt. 3)	Pt. #2	625.00	100.18	632.98	+20.89
		Pt. #3	-631.38	432.29	765.19	
Day 17	UR 1st molar (Pt. 3)	Pt. #2				
		Pt. #3				

Table F1: Rat F1 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat F1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movement of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

Figure F1: Rat F1 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

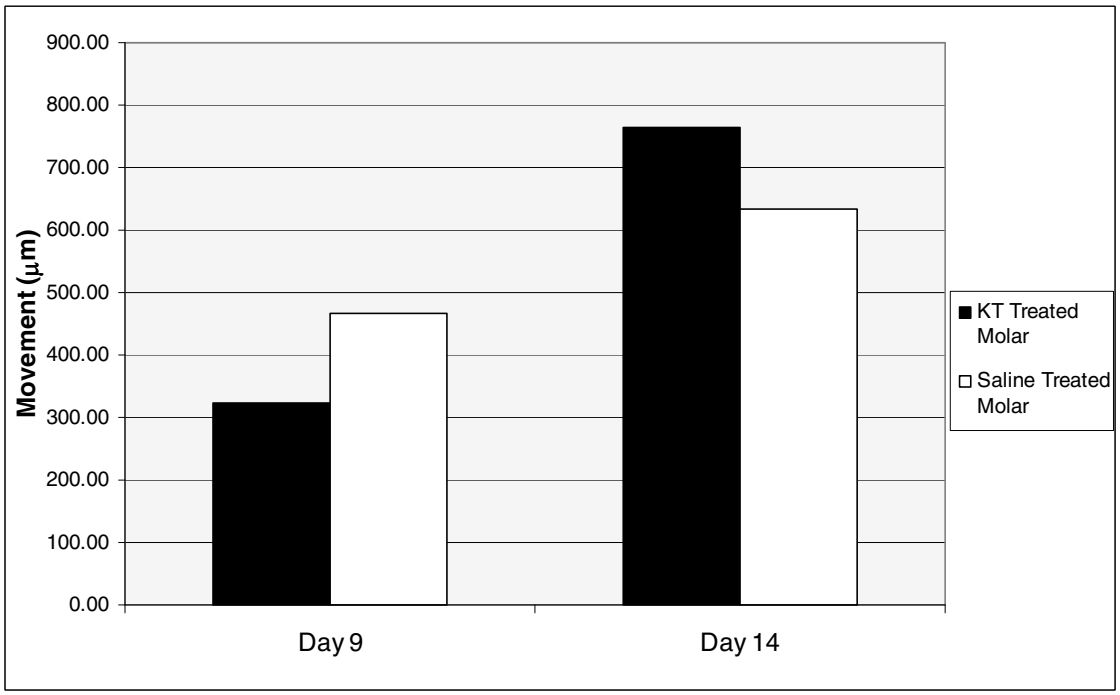

Figure F2: Time-course movements of KT and saline treated first molars during the expansion phase in rat F1. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX G

SUMMARY OF RAT F2 EXPANSION PHASE DATA

Rat F2 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UL 1st molar (Pt. 2)	Pt. #2	241.12	244.47	343.37	-27.96
		Pt. #3	-320.04	353.24	476.65	
Day 9	UL 1st molar (Pt. 2)	Pt. #2	600.57	383.26	712.44	+7.44
		Pt. #3	-380.60	542.98	663.08	
Day 13	UL 1st molar (Pt. 2)	Pt. #2				
		Pt. #3				
Day 18	UL 1st molar (Pt. 2)	Pt. #2	767.98	311.38	828.71	-15.61
		Pt. #3	-942.14	276.93	982.00	

Table G1: Rat F2 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat F2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movement of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

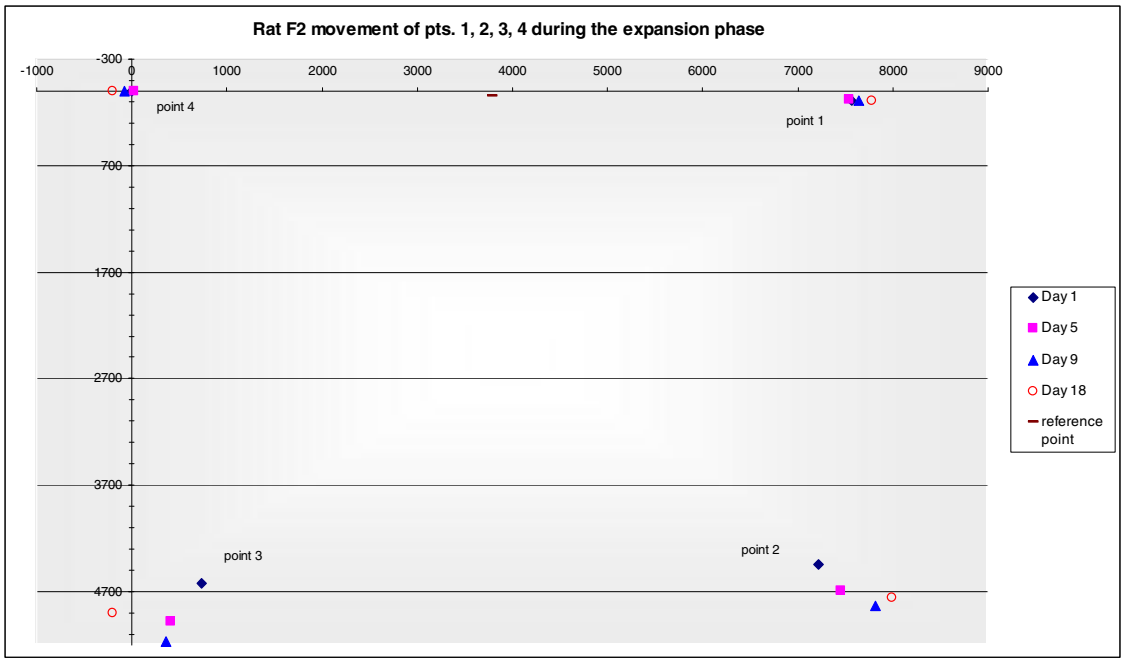

Figure G1: Rat F2 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

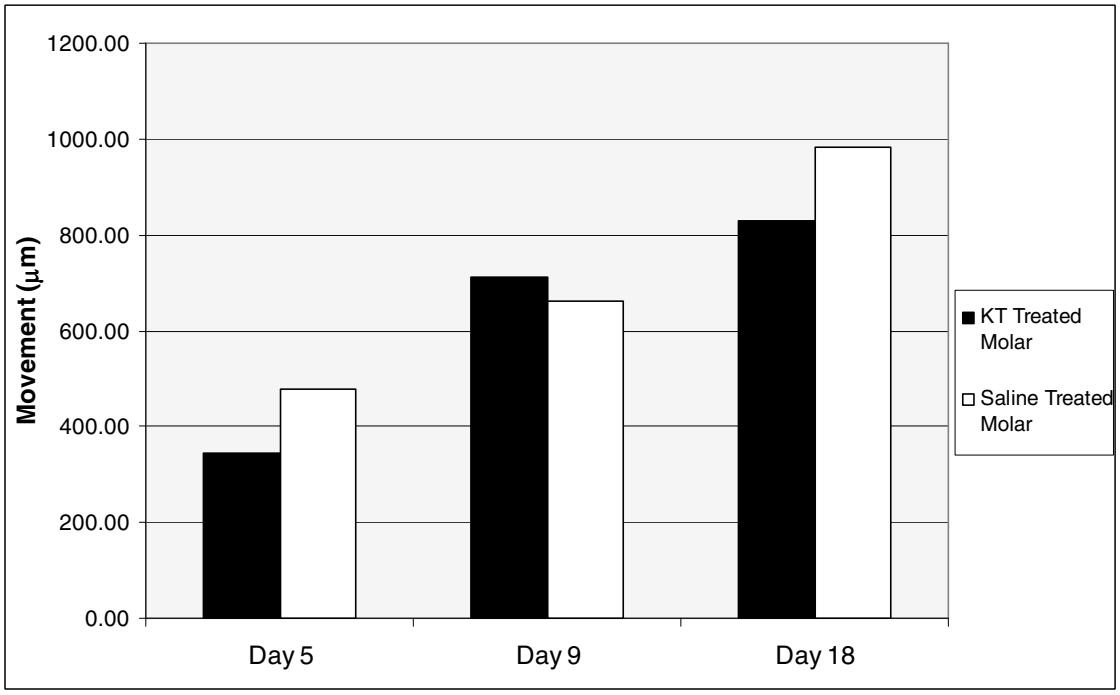

Figure G2: Time-course movements of KT and saline treated first molars during the expansion phase in rat F2. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX H

SUMMARY OF RAT G1 EXPANSION PHASE DATA

Rat G1 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UR 1st molar (Pt. 3)	Pt. #2				
		Pt. #3				
Day 9	UR 1st molar (Pt. 3)	Pt. #2				
		Pt. #3				
Day 13	UR 1st molar (Pt. 3)	Pt. #2				
		Pt. #3				
Day 17	UR 1st molar (Pt. 3)	Pt. #2	616.48	123.69	628.77	+23.71
		Pt. #3	-730.14	268.22	777.84	

Table H1: Rat G1 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat G1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movement of pts. 2 and 3 was measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movement of the KT treated tooth was calculated relative to the saline treated tooth.

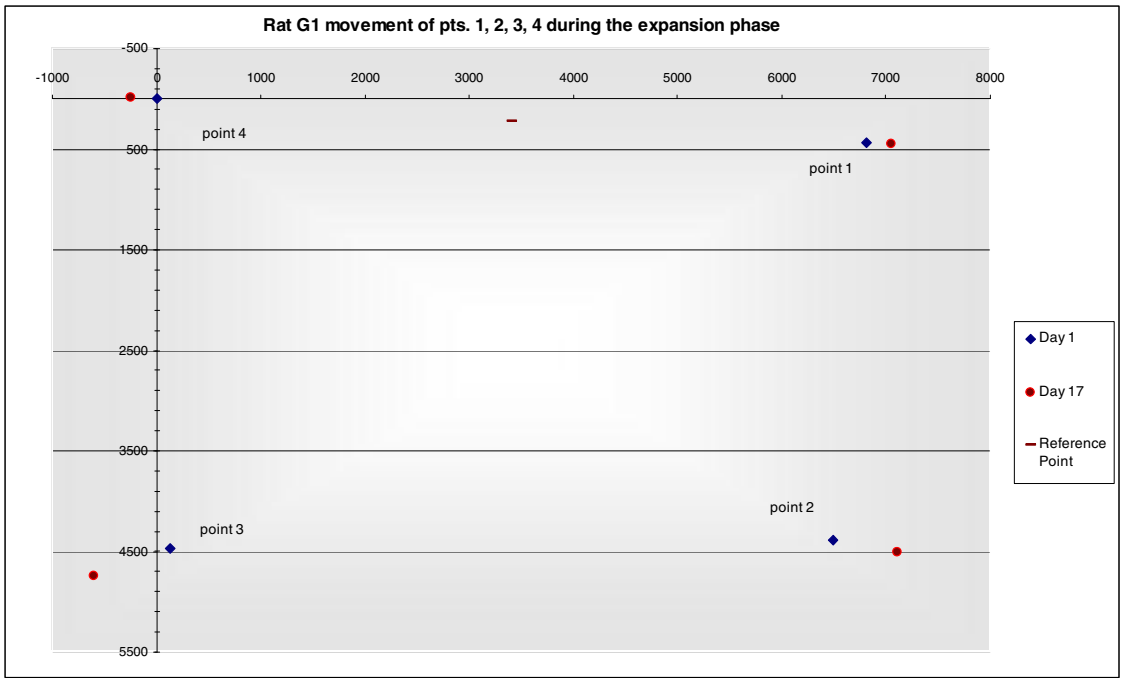

Figure H1: Rat G1 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

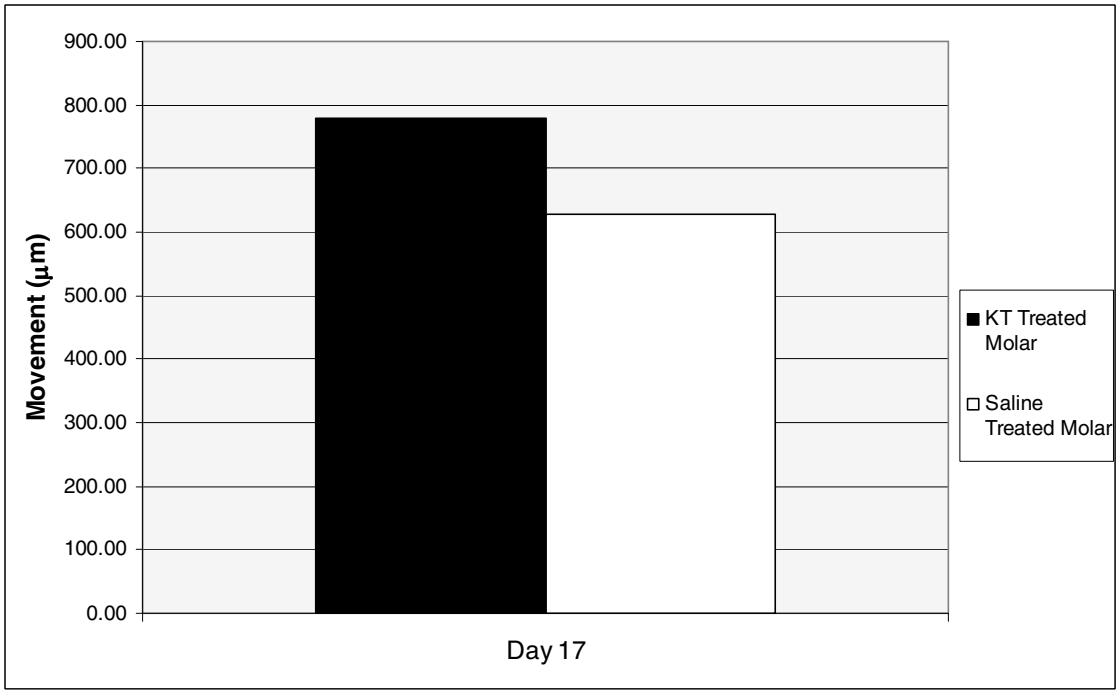

Figure H2: Time-course movements of KT and saline treated first molars during the expansion phase in rat G1. The distances (µm) moved by KT treated tooth (*black bar*) and by the saline treated tooth (*white bar*) are given at day 17.

APPENDIX I

SUMMARY OF RAT G2 EXPANSION PHASE DATA

Rat G2 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UL 1st molar (Pt. 2)	Pt. #2	242.11	218.82	326.34	-42.29
		Pt. #3	-564.81	-28.48	565.53	
Day 9	UL 1st molar (Pt. 2)	Pt. #2	458.03	202.06	500.62	+6.38
		Pt. #3	-457.72	109.42	470.61	
Day 13	UL 1st molar (Pt. 2)	Pt. #2	462.80	76.73	469.11	+21.94
		Pt. #3	-380.29	58.21	384.72	
Day 17	UL 1st molar (Pt. 2)	Pt. #2	766.65	396.90	863.30	+12.69
		Pt. #3	-715.36	274.11	766.08	

Table I1: Rat G2 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat G2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movement of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

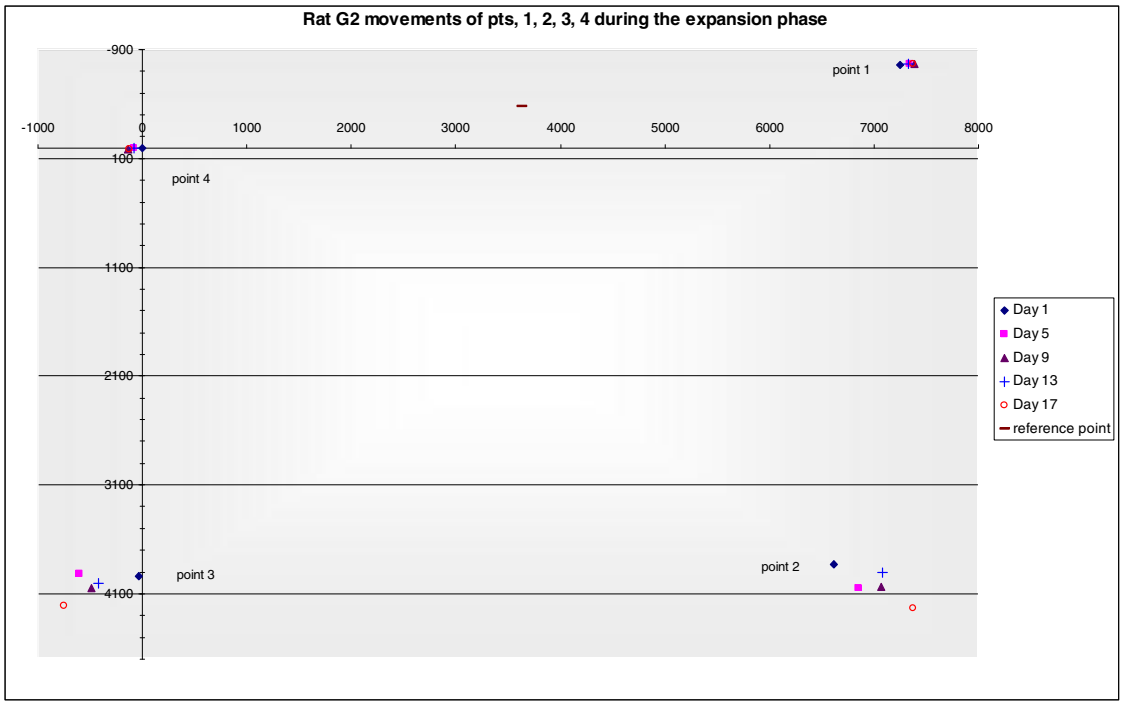

Figure I1: Rat G2 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

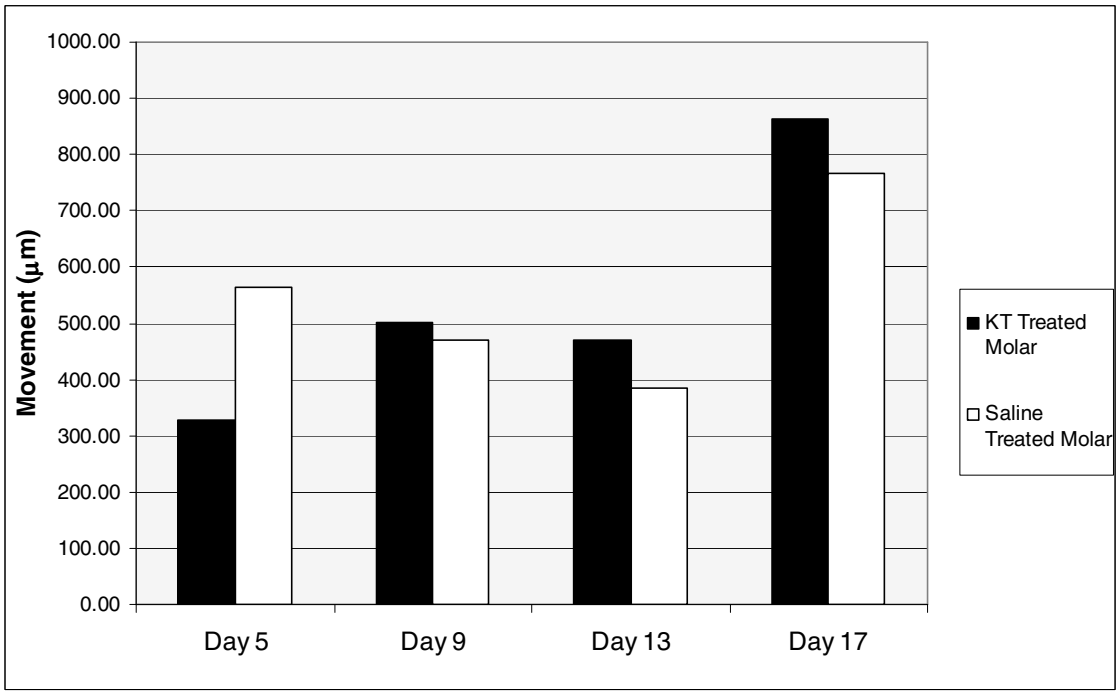

Figure I2: Time-course movements of KT and saline treated first molars during the expansion phase in rat G2. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX J

SUMMARY OF RAT H1 EXPANSION PHASE DATA

Rat H1 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UR 1st molar (Pt. 3)	Pt. #2				
		Pt. #3				
Day 9	UR 1st molar (Pt. 3)	Pt. #2	176.00	260.29	314.21	+30.60
		Pt. #3	-390.39	126.42	410.35	
Day 13	UR 1st molar (Pt. 3)	Pt. #2	352.02	234.18	422.80	+14.9478085
		Pt. #3	-405.56	267.80	486.00	
Day 17	UR 1st molar (Pt. 3)	Pt. #2	234.20	405.99	468.70	+67.02
		Pt. #3	-754.13	209.92	782.80	

Table J1: Rat H1 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat H1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movement of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

Figure J1: Rat H1 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

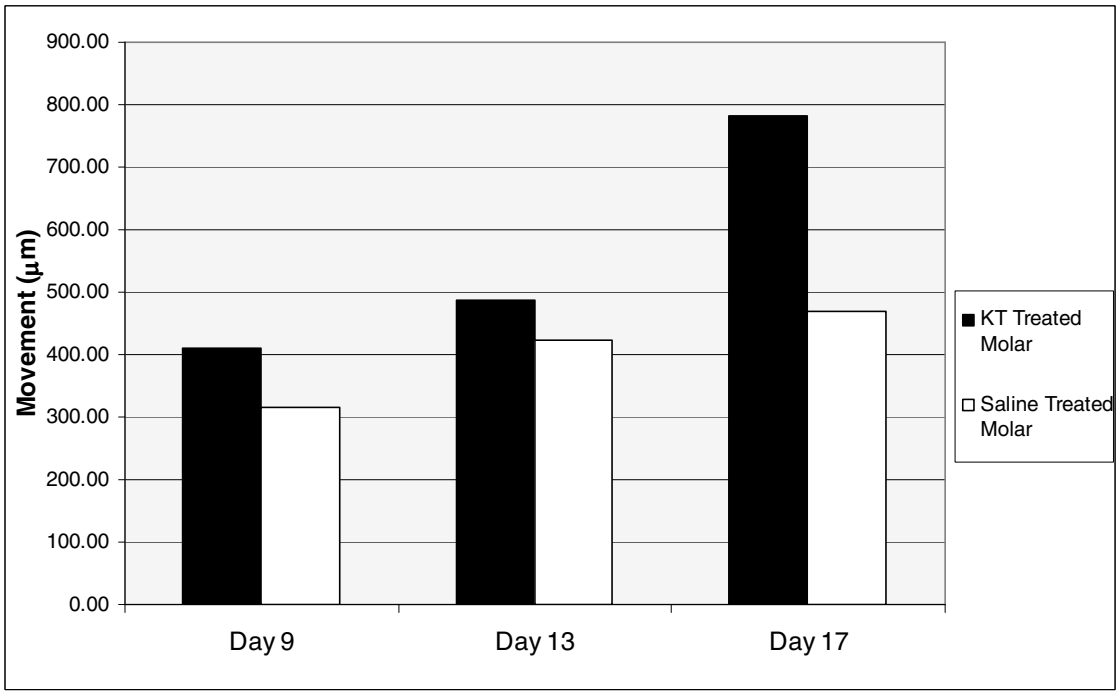

Figure J2: Time-course movements of KT and saline treated first molars during the expansion phase in rat H1. The distances (m) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX K

SUMMARY OF RAT H2 EXPANSION PHASE DATA

Rat C2 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UL 1st molar (Pt. 2)	Pt. #2	360.40	255.34	441.68	-6.19
		Pt. #3	-458.23	108.19	470.83	
Day 9	UL 1st molar (Pt. 2)	Pt. #2	715.14	86.81	720.39	-8.80
		Pt. #3	-769.46	178.53	789.90	
Day 13	UL 1st molar (Pt. 2)	Pt. #2	895.91	-64.89	898.26	+6.07
		Pt. #3	-835.80	115.31	843.71	
Day 16	UL 1st molar (Pt. 2)	Pt. #2	588.31	274.56	649.22	-83.90
		Pt. #3	-1189.89	97.93	1193.91	

Table K1: Rat H2 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat H2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movement of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

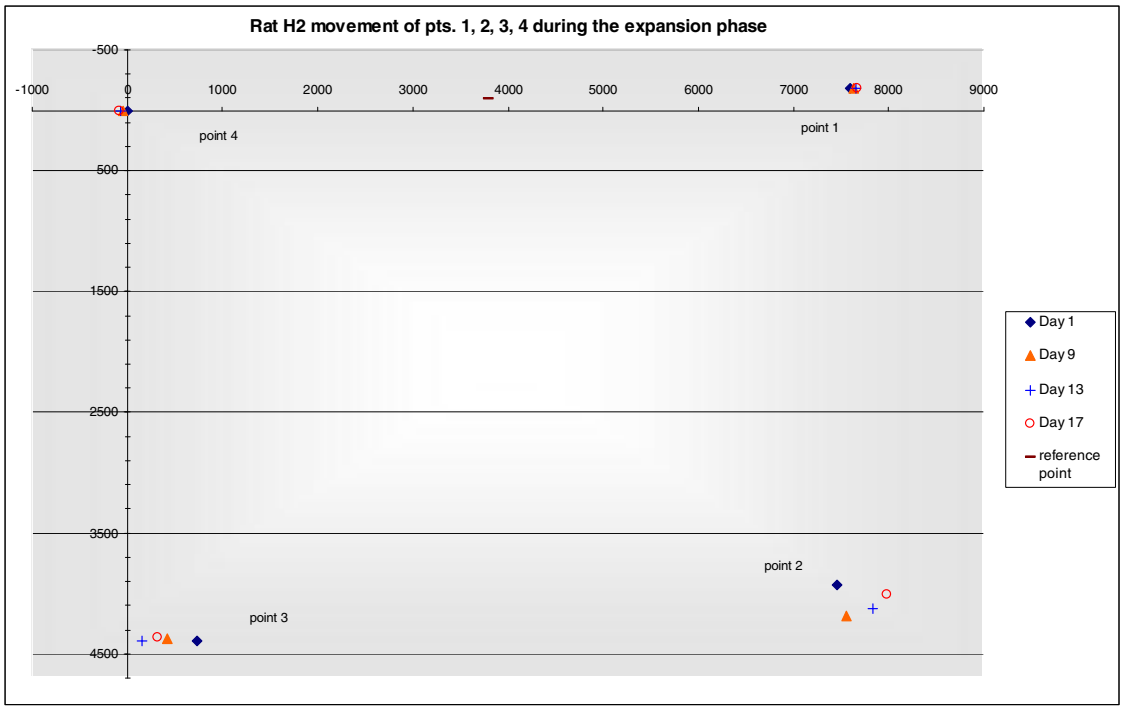

Figure K1: Rat H2 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

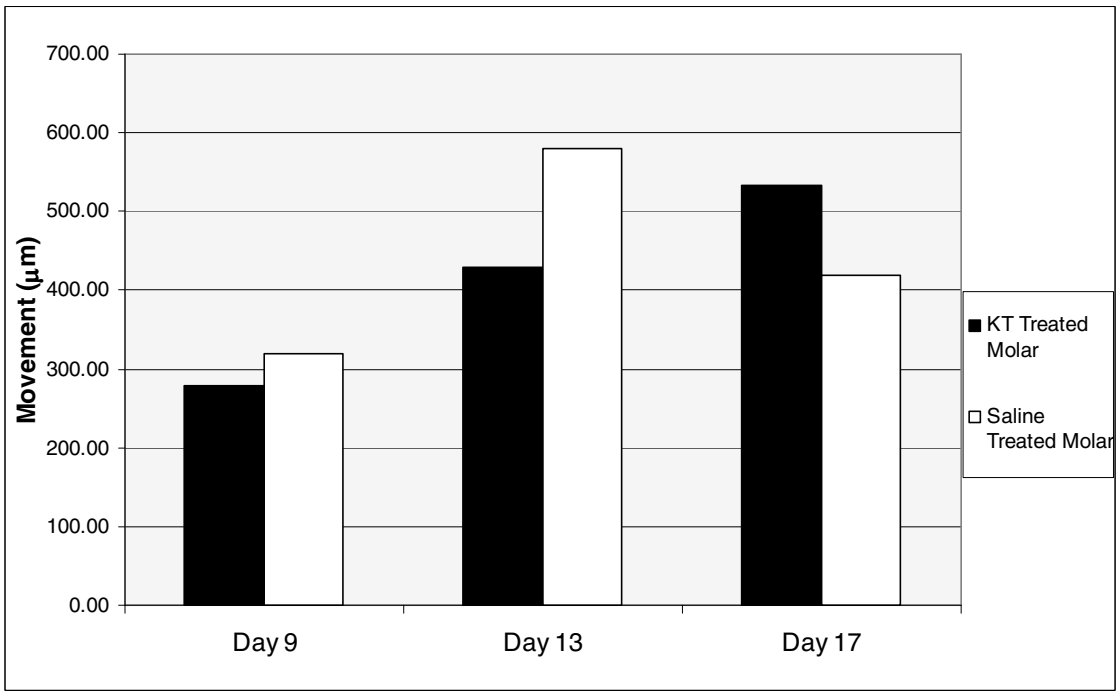

Figure K2: Time-course movements of KT and saline treated first molars during the expansion phase in rat H2. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX L

SUMMARY OF RAT D1 EXPANSION PHASE DATA

Rat D1 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UR 1st molar (Pt. 3)	Pt. #2	258.54	170.47	309.68	+64.81
		Pt. #3	-509.19	35.00	510.39	
Day 9	UR 1st molar (Pt. 3)	Pt. #2	300.56	-70.99	308.83	+47.33
		Pt. #3	-406.87	-203.66	454.99	
Day 13	UR 1st molar (Pt. 3)	Pt. #2	612.51	-54.55	614.93	-13.03
		Pt. #3	-534.10	27.46	534.81	

Table K1: Rat D1 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat D1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movement of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

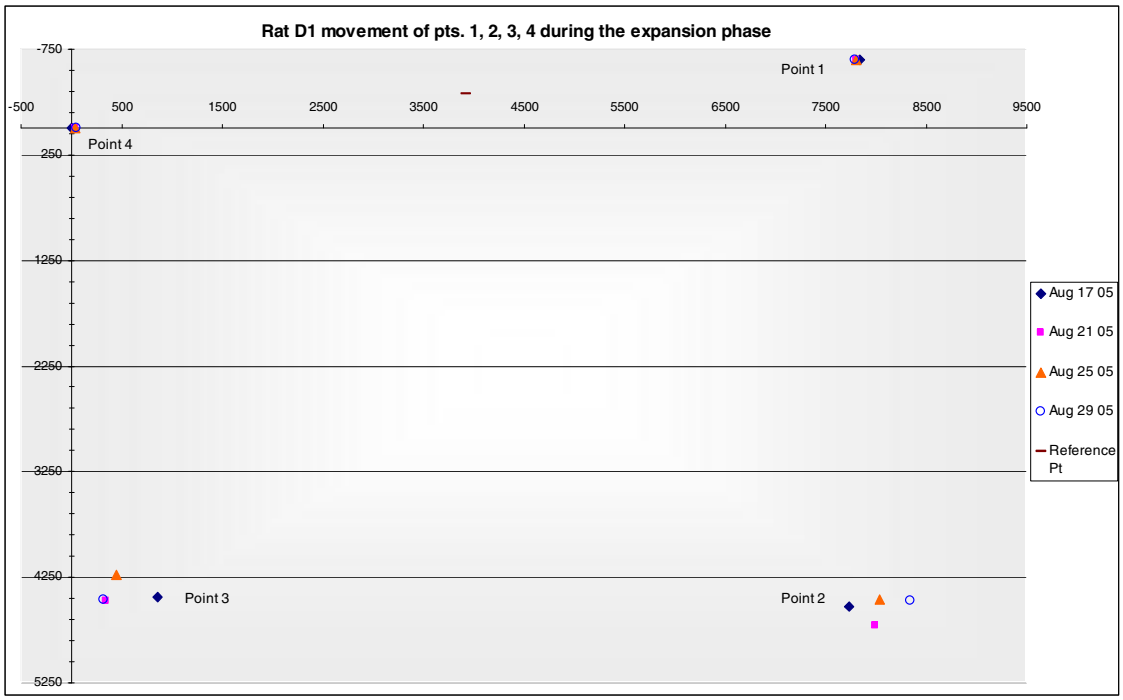

Figure K1: Rat D1 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

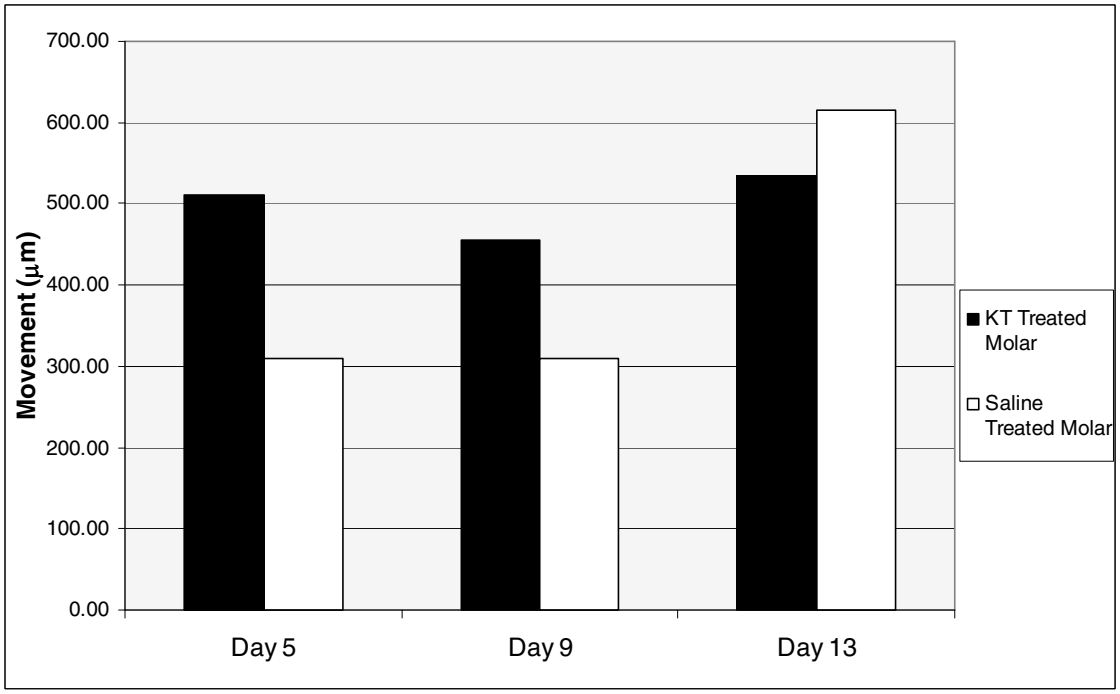

Figure K2: Time-course movements of KT and saline treated first molars during the expansion phase in rat D1. The distances (μm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX M

SUMMARY OF RAT E2 EXPANSION PHASE DATA

Rat E2 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UL 1st molar (Pt. 2)	Pt. #2	143.85	131.35	194.80	-47.90
		Pt. #3	-366.47	74.20	373.91	
Day 9	UL 1st molar (Pt. 2)	Pt. #2	284.04	217.16	357.55	-32.14
		Pt. #3	-499.00	169.19	526.91	
Day 13	UL 1st molar (Pt. 2)	Pt. #2	432.02	-59.74	436.13	+24.01
		Pt. #3	-351.69	-1.26	351.69	

Table M1: Rat E2 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat E2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movement of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

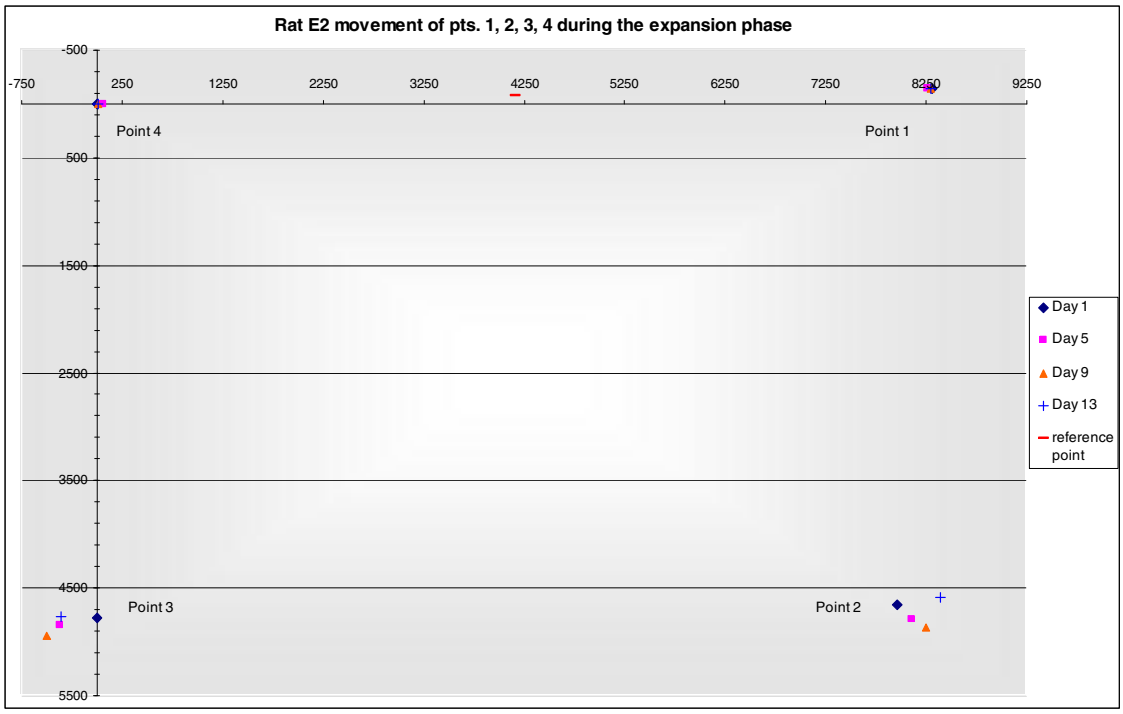

Figure M1: Rat E2 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

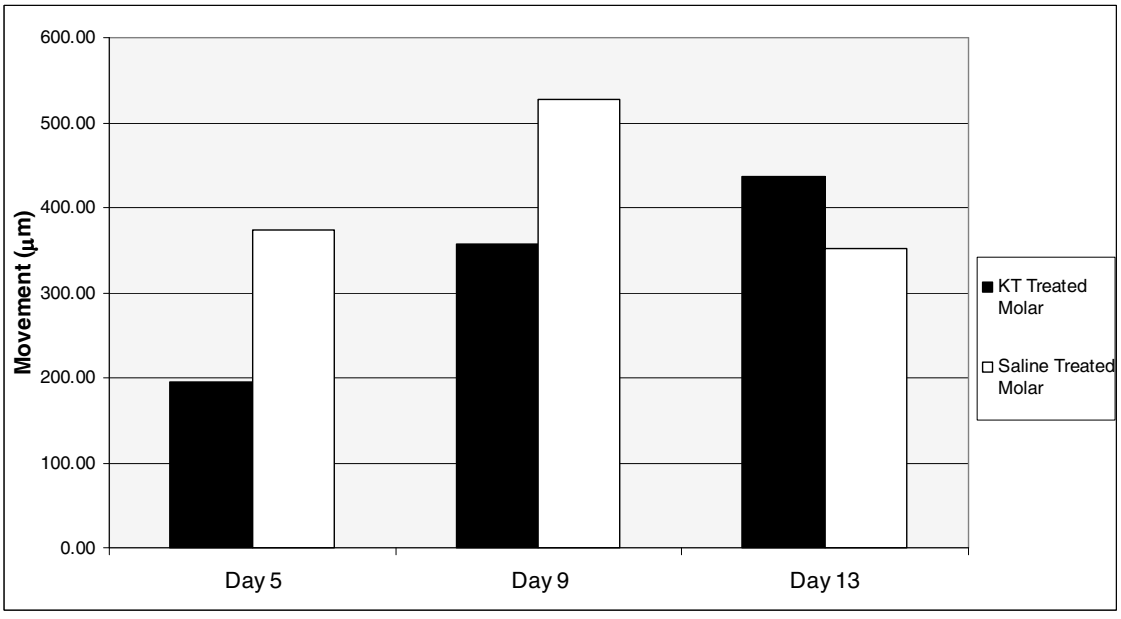

Figure M2: Time-course movements of KT and saline treated first molars during the expansion phase in rat E2. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX N

SUMMARY OF RAT F1 SECONDEXPANSION PHASE DATA

Rat F1 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UR 1st molar (Pt. 3)	Pt. #2	268.53	53.68	273.85	-46.25
		Pt. #3	-147.05	-6.75	147.20	
Day 9	UR 1st molar (Pt. 3)	Pt. #2	209.06	134.16	248.41	-43.16
		Pt. #3	-128.38	58.79	141.21	
Day 13	UR 1st molar (Pt. 3)	Pt. #2	422.71	248.42	490.30	-67.48
		Pt. #3	-111.53	113.94	159.44	

Table N1: Rat F1 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat F1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movement of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

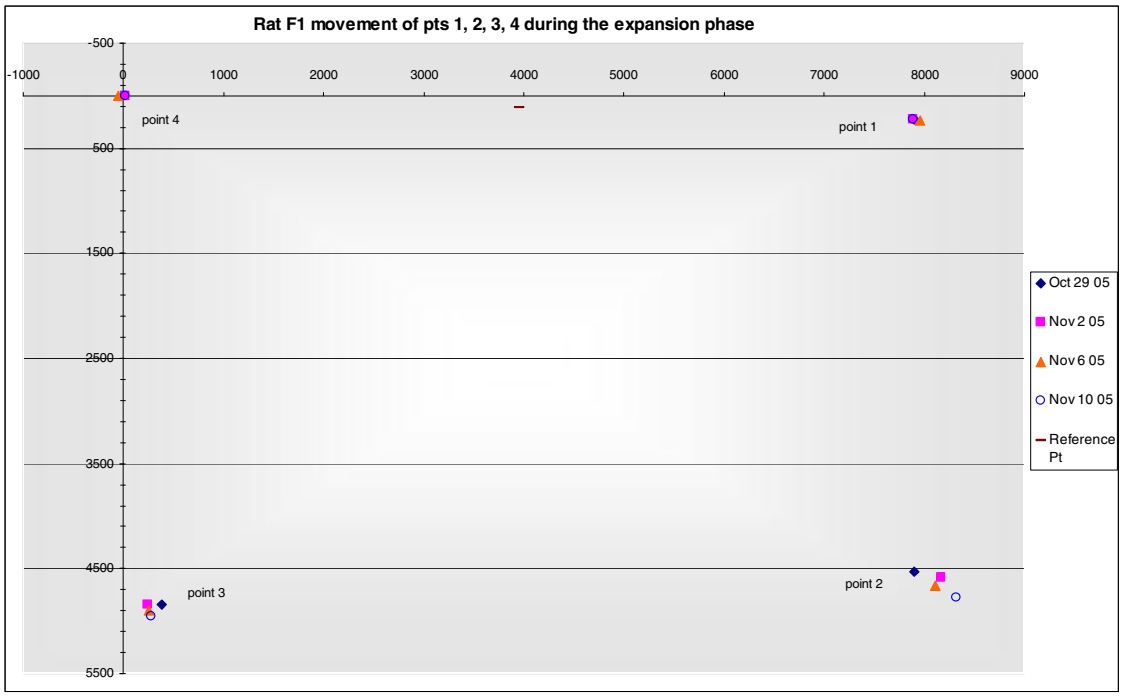

Figure N1: Rat F1 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

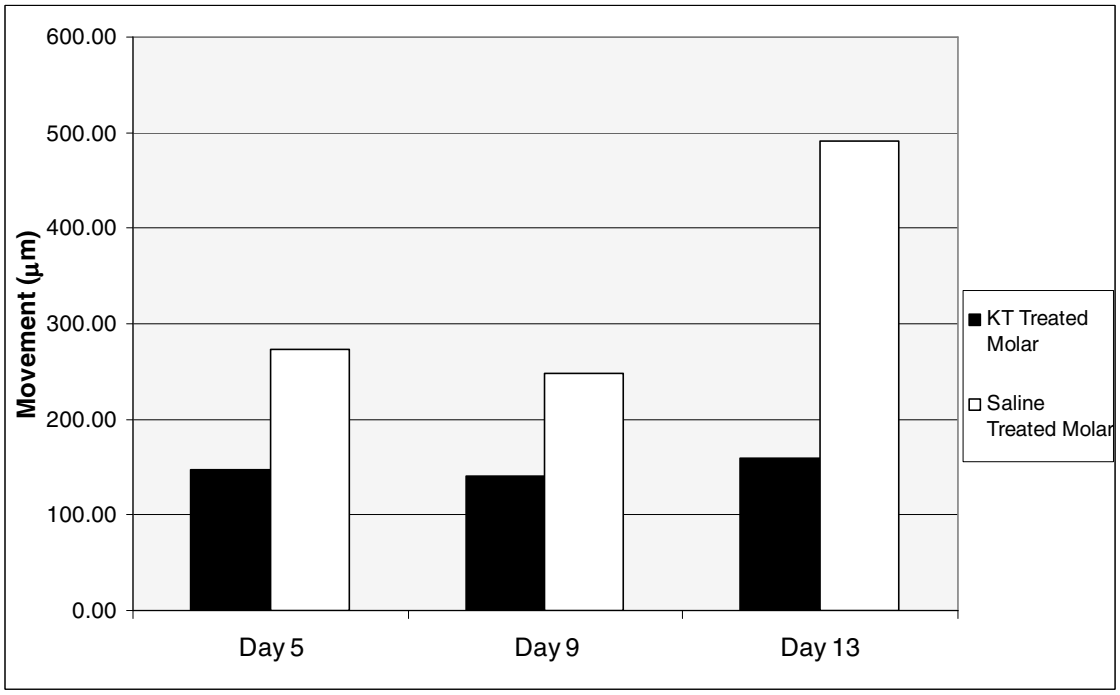

Figure N2: Time-course movements of KT and saline treated first molars during the expansion phase in rat F1. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX O

SUMMARY OF RAT F2 SECONDEXPANSION PHASE DATA

Rat F2 tooth movement measurements during expansion phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UL 1st molar (Pt. 2)	Pt. #2				
		Pt. #3				
Day 9	UL 1st molar (Pt. 2)	Pt. #2	176.26	206.38	271.41	-21.93
		Pt. #3	-211.35	276.04	347.66	
Day 13	UL 1st molar (Pt. 2)	Pt. #2	128.36	66.01	144.34	-45.25
		Pt. #3	-262.84	-20.39	263.63	

Table O1: Rat F2 tooth movement measurements during expansion phase. Reported in the table are the time points when the appliances were retained in the rat's mouth. In rat F2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movements of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

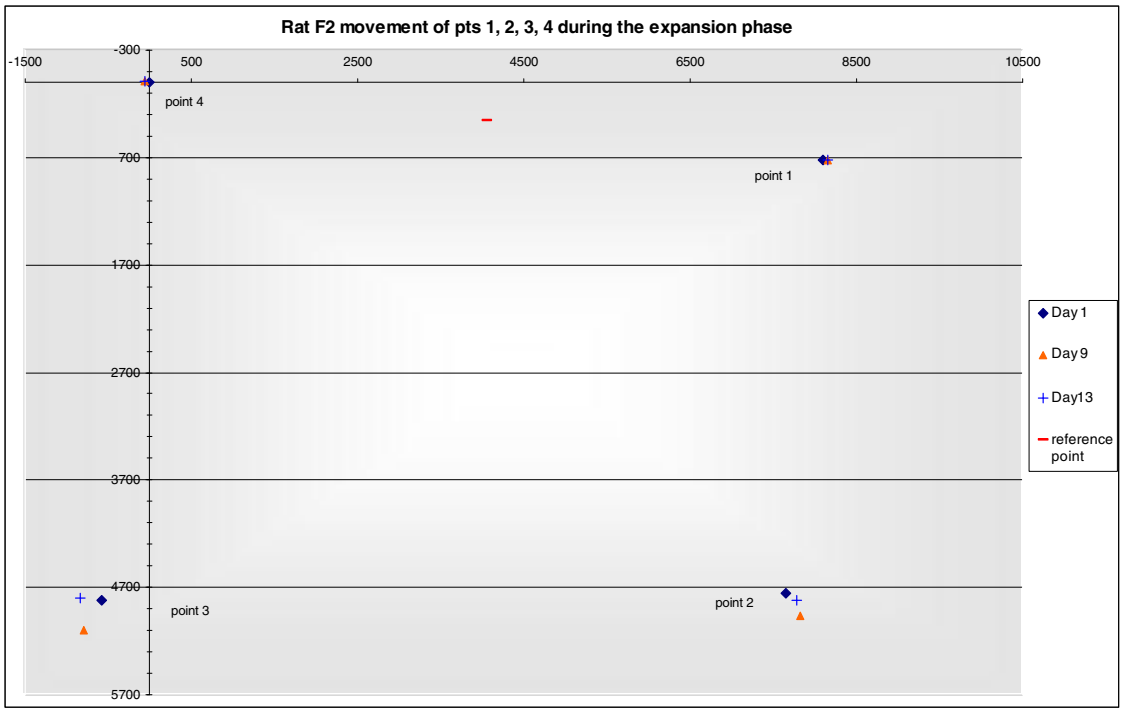

Figure O1: Rat F2 movement of pts. 1, 2, 3, 4 during the expansion phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

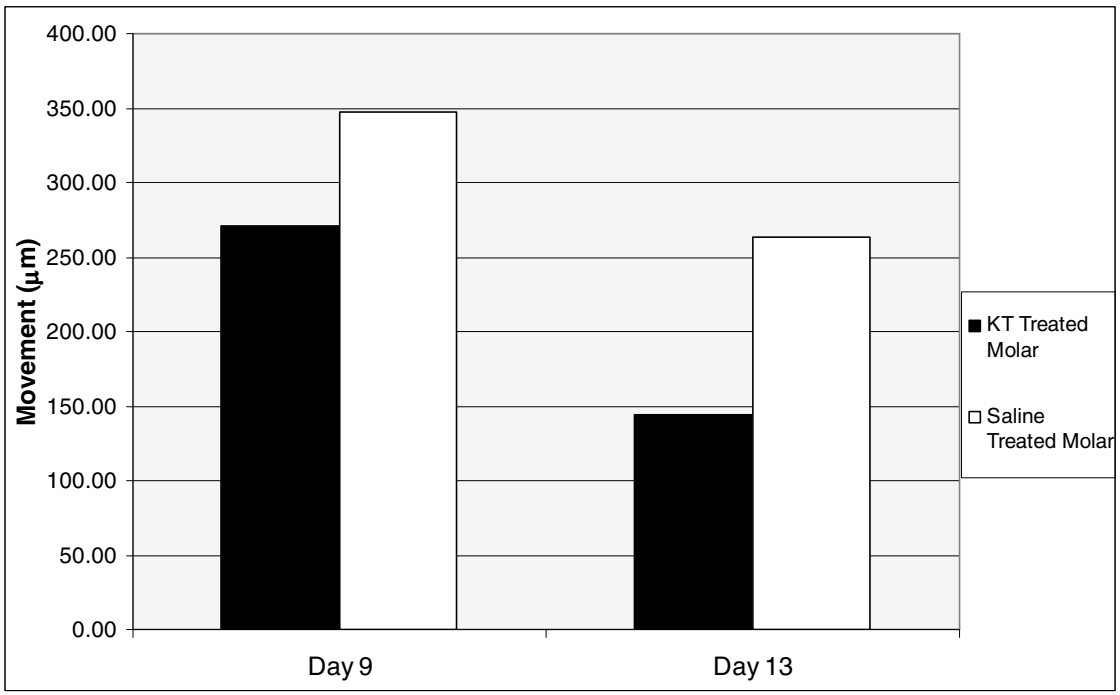

Figure O2: Time-course movements of KT and saline treated first molars during the expansion phase in rat F2. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX P

SUMMARY OF RAT C1 RELAPSE PHASE DATA

Rat C1 tooth movement measurements during relapse phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UR 1st molar (Pt. 3)	Pt. #2	-10.34	210.37	210.63	+1.29
		Pt. #3	-196.57	82.91	213.34	
Day 9	UR 1st molar (Pt. 3)	Pt. #2	-32.40	59.03	67.34	+311.13
		Pt. #3	-273.15	-45.03	276.84	
Day 13	UR 1st molar (Pt. 3)	Pt. #2	30.50	-30.67	43.26	+339.24
		Pt. #3	-117.05	-149.66	190.00	

Table P1: Rat C1 tooth movement measurements during relapse phase. Reported in the table are the time points when impressions were taken. In rat C1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movements of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

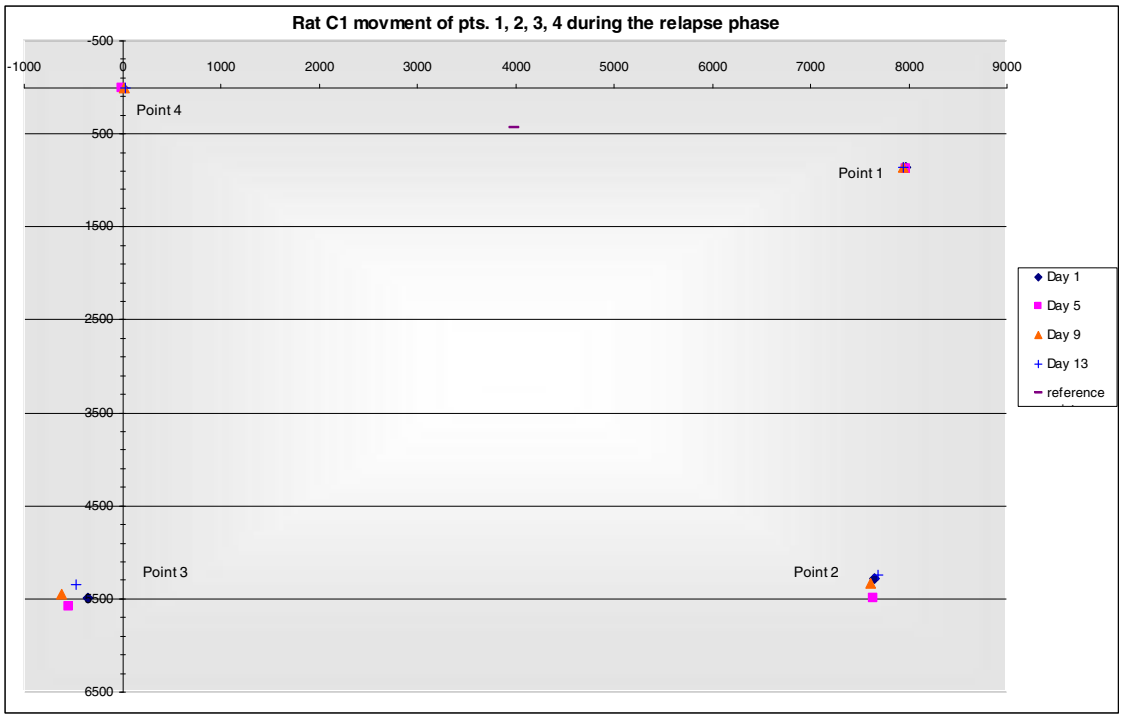

Figure P1: Rat C1 movement of pts. 1, 2, 3, 4 during the relapse phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

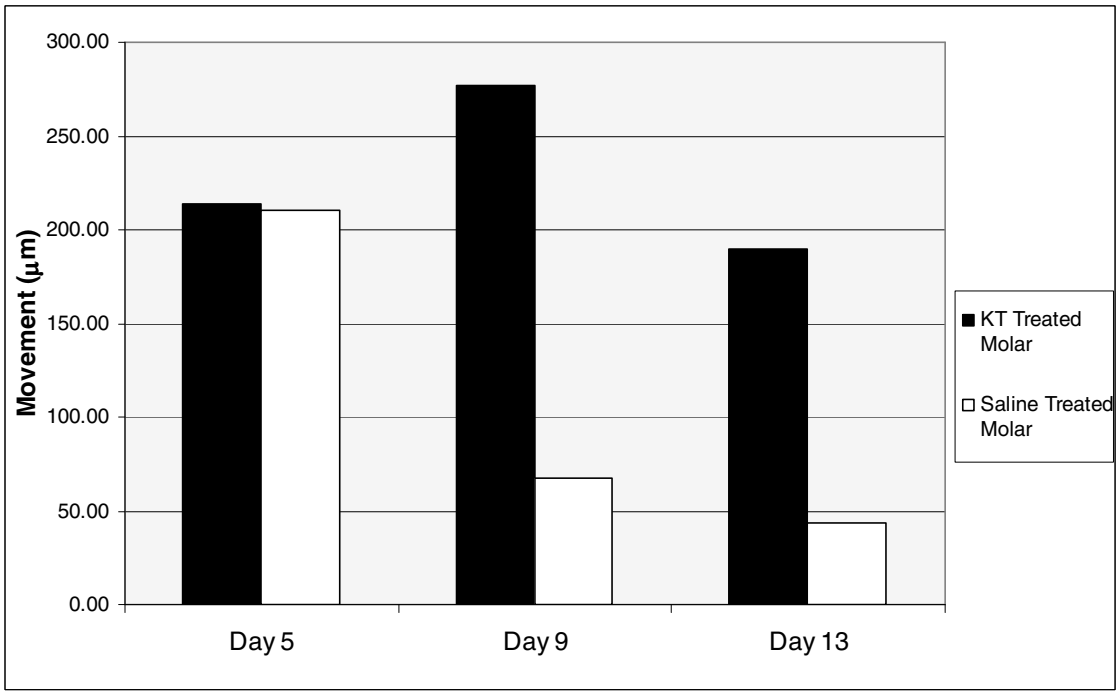

Figure P2: Time-course movements of KT and saline treated first molars during the relapse phase in rat C1. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX Q

SUMMARY OF RAT C2 RELAPSE PHASE DATA

Rat C2 tooth movement measurements during relapse phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UL 1st molar (Pt. 2)	Pt. #2	74.49	107.63	130.89	-56.52
		Pt. #3	210.15	215.51	301.01	
Day 9	UL 1st molar (Pt. 2)	Pt. #2	-46.34	44.55	64.28	-73.09
		Pt. #3	69.83	228.42	238.86	
Day 13	UL 1st molar (Pt. 2)	Pt. #2	36.20	-253.61	256.18	-10.30
		Pt. #3	272.07	-86.85	285.60	

Table Q1: Rat C2 tooth movement measurements during relapse phase. Reported in the table are the time points when impressions were taken. In rat C2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movements of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

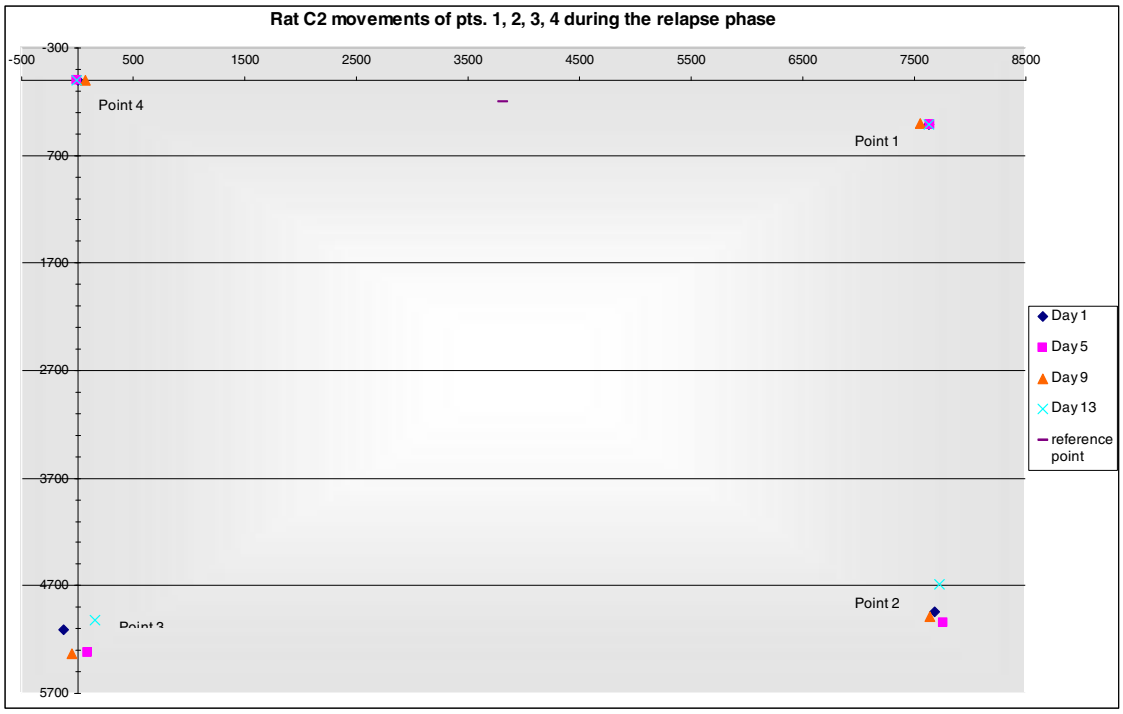

Figure Q1: Rat C2 movement of pts. 1, 2, 3, 4 during the relapse phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

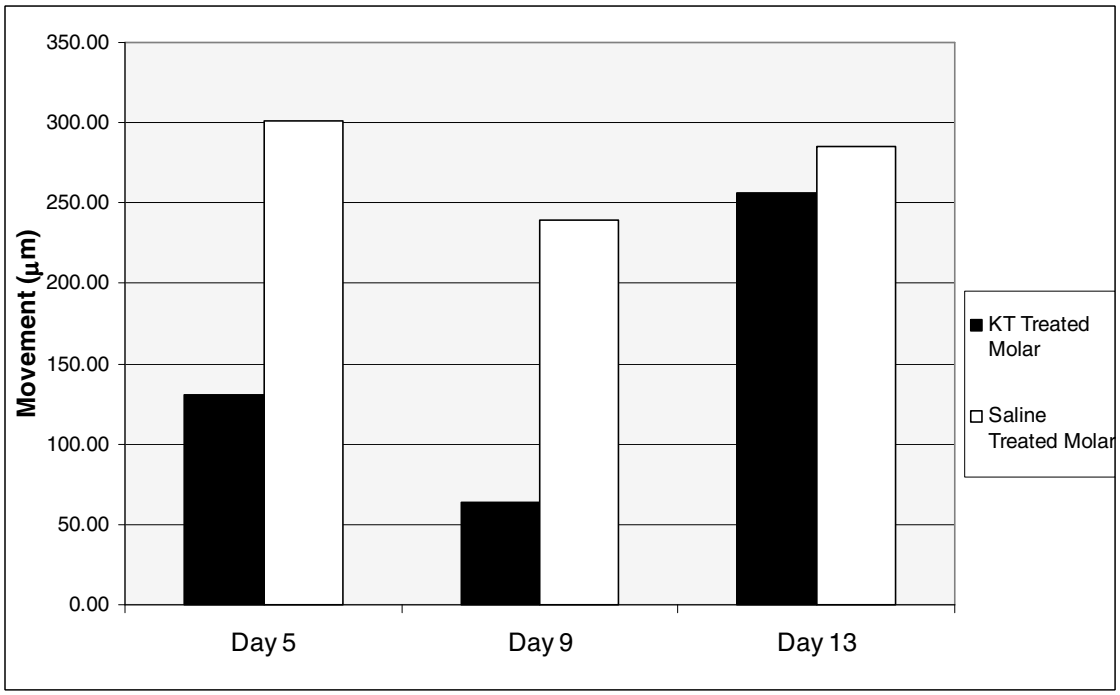

Figure Q2: Time-course movements of KT and saline treated first molars during the relapse phase in rat C2. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX R

SUMMARY OF RAT D2 RELAPSE PHASE DATA

Rat D2 tooth movement measurements during relapse phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UL 1st molar (Pt. 2)	Pt. #2	2.96	9.97	10.40	-93.86
		Pt. #3	-57.06	159.41	169.32	
Day 9	UL 1st molar (Pt. 2)	Pt. #2	87.42	-118.75	147.46	-5.42
		Pt. #3	-9.11	155.64	155.90	
Day 13	UL 1st molar (Pt. 2)	Pt. #2	107.42	-275.46	295.66	+143.34
		Pt. #3	3.33	-121.45	121.50	

Table R1: Rat D2 tooth movement measurements during relapse phase. Reported in the table are the time points when impressions were taken. In rat D2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movements of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

Figure R1: Rat D2 movement of pts. 1, 2, 3, 4 during the relapse phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

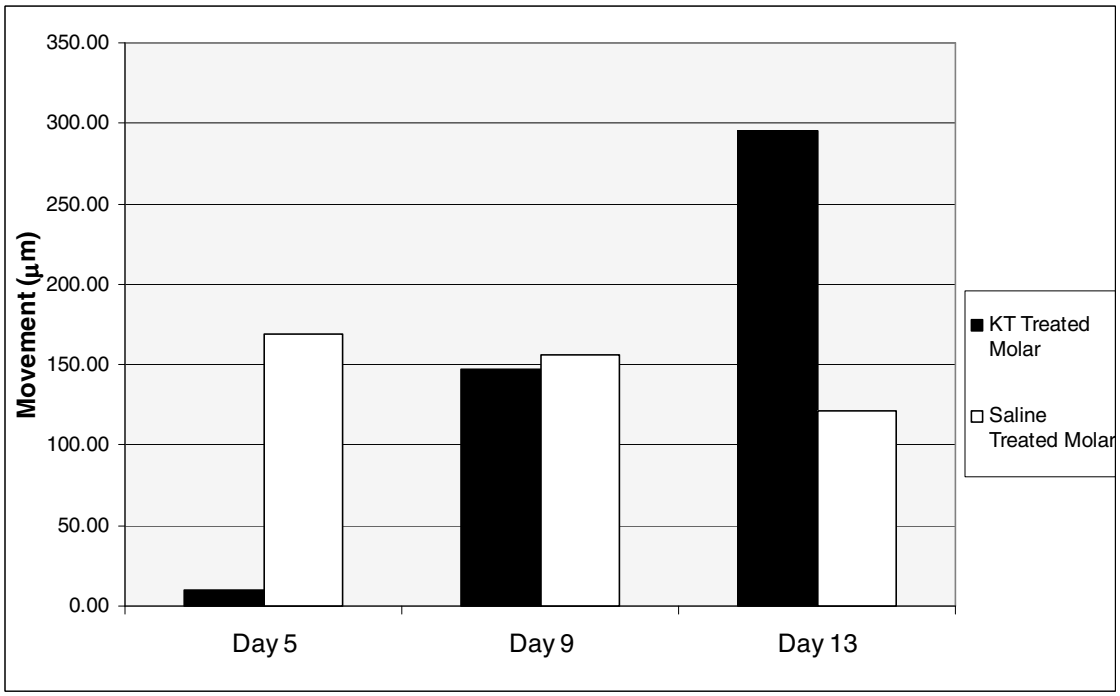

Figure R2: Time-course movements of KT and saline treated first molars during the relapse phase in rat D2. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX S

SUMMARY OF RAT E1 RELAPSE PHASE DATA

Rat E1 tooth movement measurements during relapse phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UR 1st molar (Pt. 3)	Pt. #2	75.53	-118.78	140.76	+20.68
		Pt. #3	162.37	-49.92	169.87	
Day 9	UR 1st molar (Pt. 3)	Pt. #2	140.24	-173.40	223.01	-9.09
		Pt. #3	120.18	-163.27	202.73	
Day 13	UR 1st molar (Pt. 3)	Pt. #2	134.04	-153.60	203.86	+7.13
		Pt. #3	162.96	-145.40	218.40	

Table S1: Rat E1 tooth movement measurements during relapse phase. Reported in the table are the time points when impressions were taken. In rat E1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movement of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

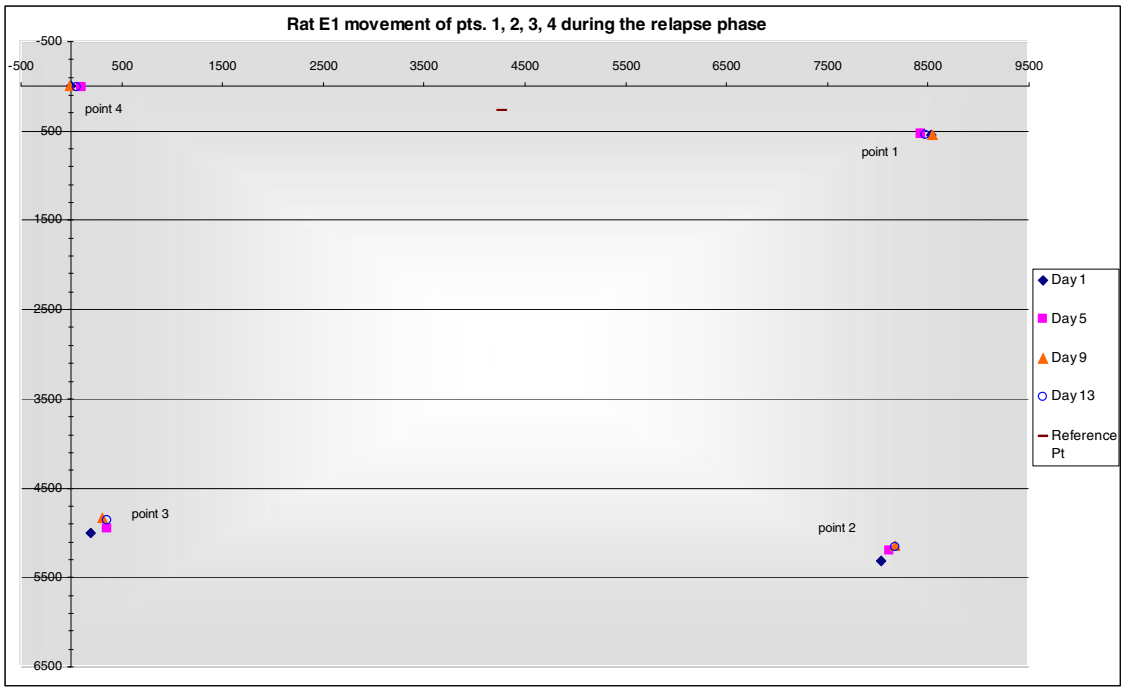

Figure S1: Rat E1 movement of pts. 1, 2, 3, 4 during the relapse phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

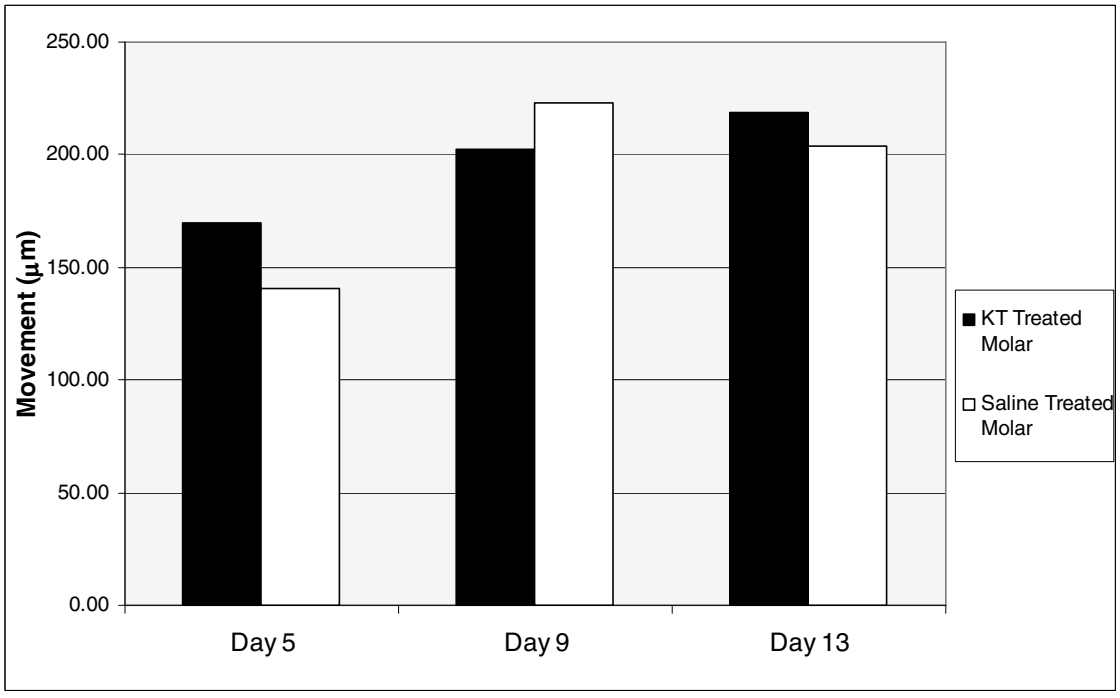

Figure S2: Time-course movements of KT and saline treated first molars during the relapse phase in rat E1. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX T

SUMMARY OF RAT G1 RELAPSE PHASE DATA

Rat G1 tooth movement measurements during relapse phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UR 1st molar (Pt. 3)	Pt. #2	-105.97	94.02	141.67	+35.50
		Pt. #3	31.47	-189.37	191.96	
Day 9	UR 1st molar (Pt. 3)	Pt. #2	-130.10	-15.42	131.01	+192.11
		Pt. #3	178.65	-338.44	382.70	
Day 13	UR 1st molar (Pt. 3)	Pt. #2	-315.84	71.53	323.83	-1.11
		Pt. #3	32.62	-318.59	320.25	

Table T1: Rat G1 tooth movement measurements during relapse phase. Reported in the table are the time points when impressions were taken. In rat G1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movements of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

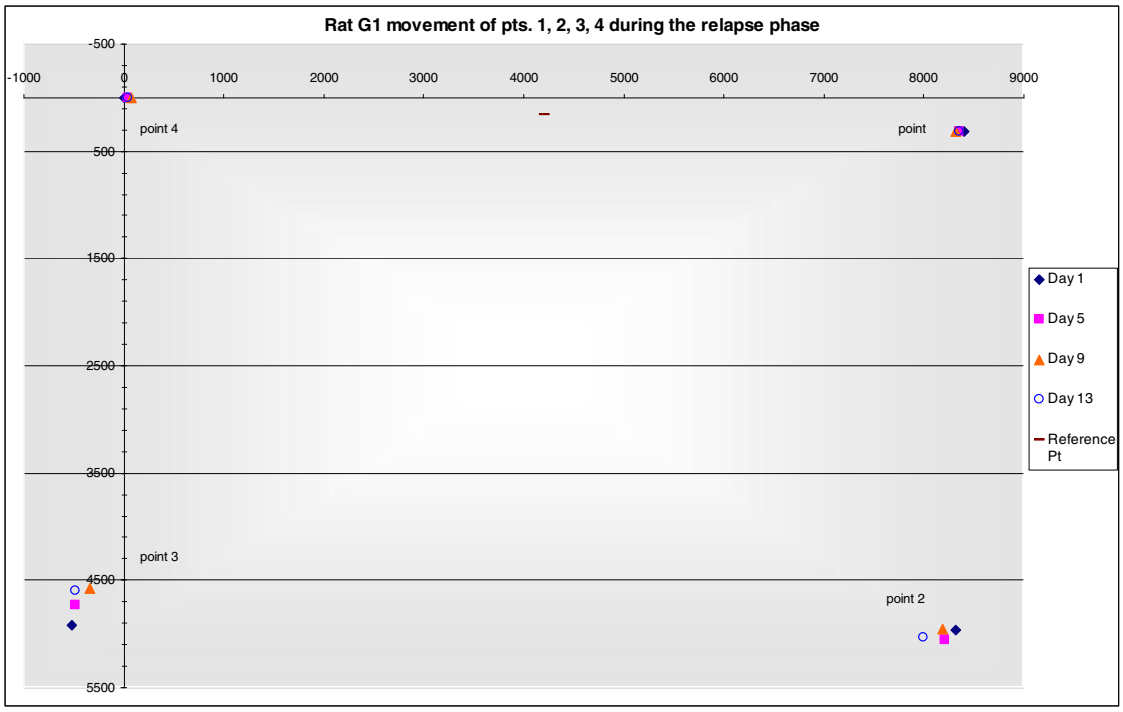

Figure T1: Rat G1 movement of pts. 1, 2, 3, 4 during the relapse phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

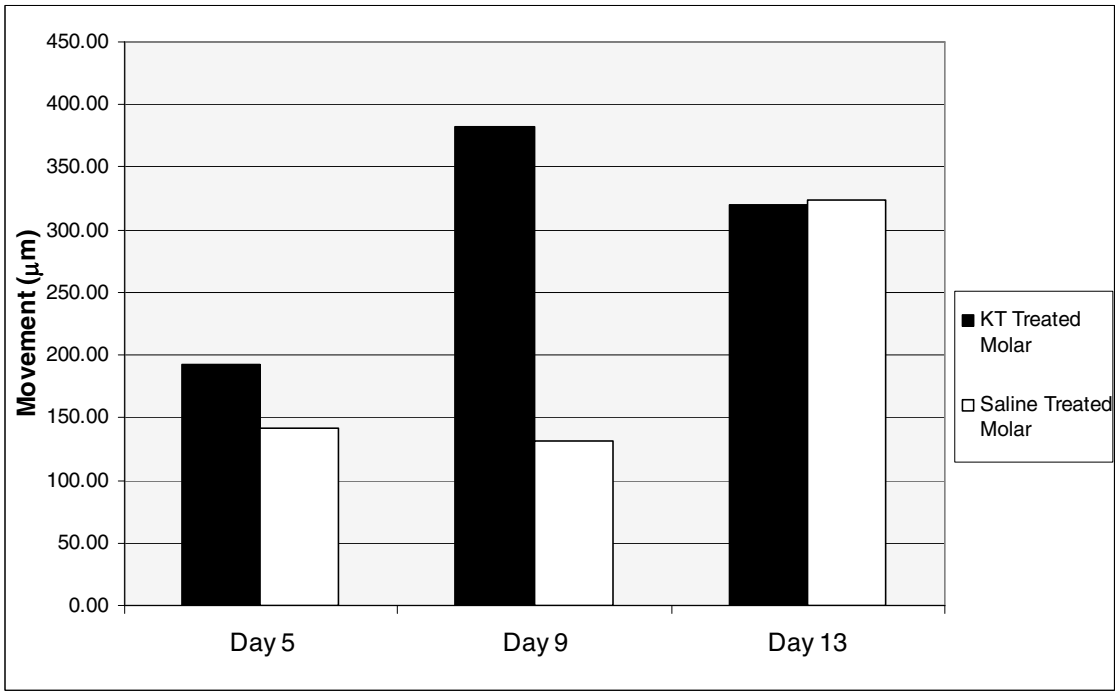

Figure T2: Time-course movements of KT and saline treated first molars during the relapse phase in rat G1. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX U

SUMMARY OF RAT G2 RELAPSE PHASE DATA

Rat G2 tooth movement measurements during relapse phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 5	UL 1st molar (Pt. 2)	Pt. #2	12.60	39.92	41.87	-63.74
		Pt. #3	98.30	-60.60	115.48	
Day 9	UL 1st molar (Pt. 2)	Pt. #2	-240.42	50.80	245.72	+106.23
		Pt. #3	51.42	-107.49	119.15	
Day 13	UL 1st molar (Pt. 2)	Pt. #2	-98.97	143.41	174.25	-2.83
		Pt. #3	177.87	22.78	179.32	

TableU1: Rat G2 tooth movement measurements during relapse phase. Reported in the table are the time points when impressions were taken. In rat G2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movements of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

Figure U1: Rat G2 movement of pts. 1, 2, 3, 4 during the relapse phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

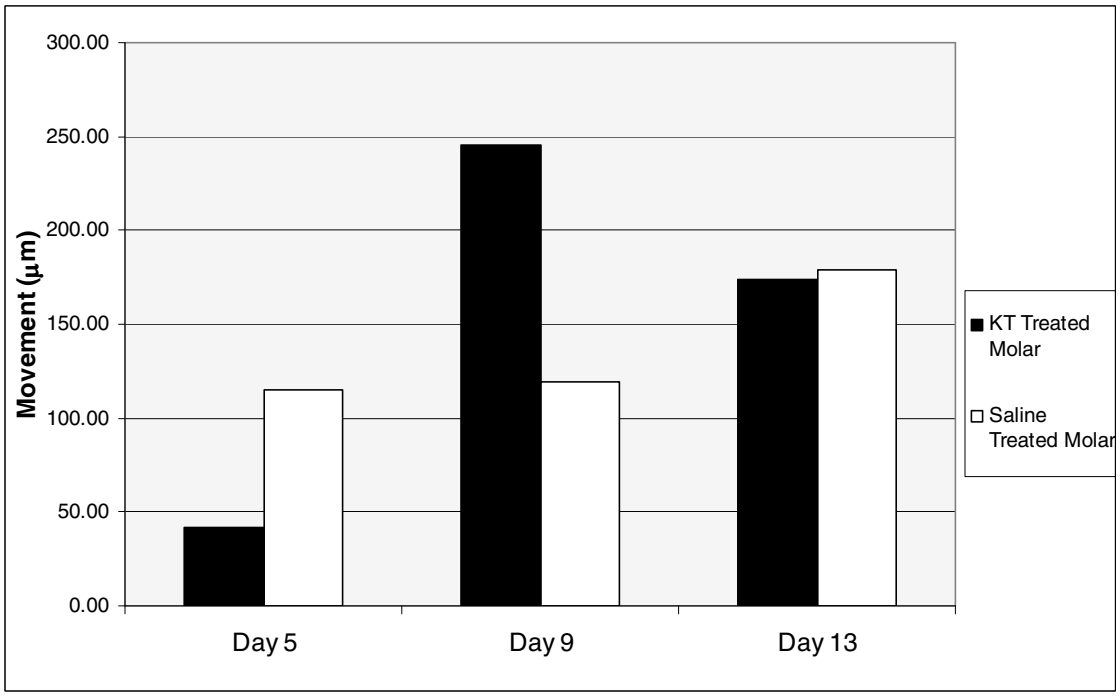

Figure U2: Time-course movements of KT and saline treated first molars during the relapse phase in rat G2. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX V

SUMMARY OF RAT H1 RELAPSE PHASE DATA

Rat H1 tooth movement measurements during relapse phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 6	UR 1st molar (Pt. 3)	Pt. #2	60.16	-211.90	220.27	+4.24
		Pt. #3	-29.60	-227.70	229.61	
Day 10	UR 1st molar (Pt. 3)	Pt. #2	53.90	-375.22	379.07	+0.10
		Pt. #3	76.41	-371.69	379.46	
Day 13	UR 1st molar (Pt. 3)	Pt. #2	67.52	-97.75	118.80	+39.43
		Pt. #3	-33.27	-162.27	165.64	

Table V1: Rat H1 tooth movement measurements during relapse phase. Reported in the table are the time points when impressions were taken. In rat H1, KT was injected near the UR first molar (pt. 3), while the UL first molar (pt. 2) was the control side. The total movements of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

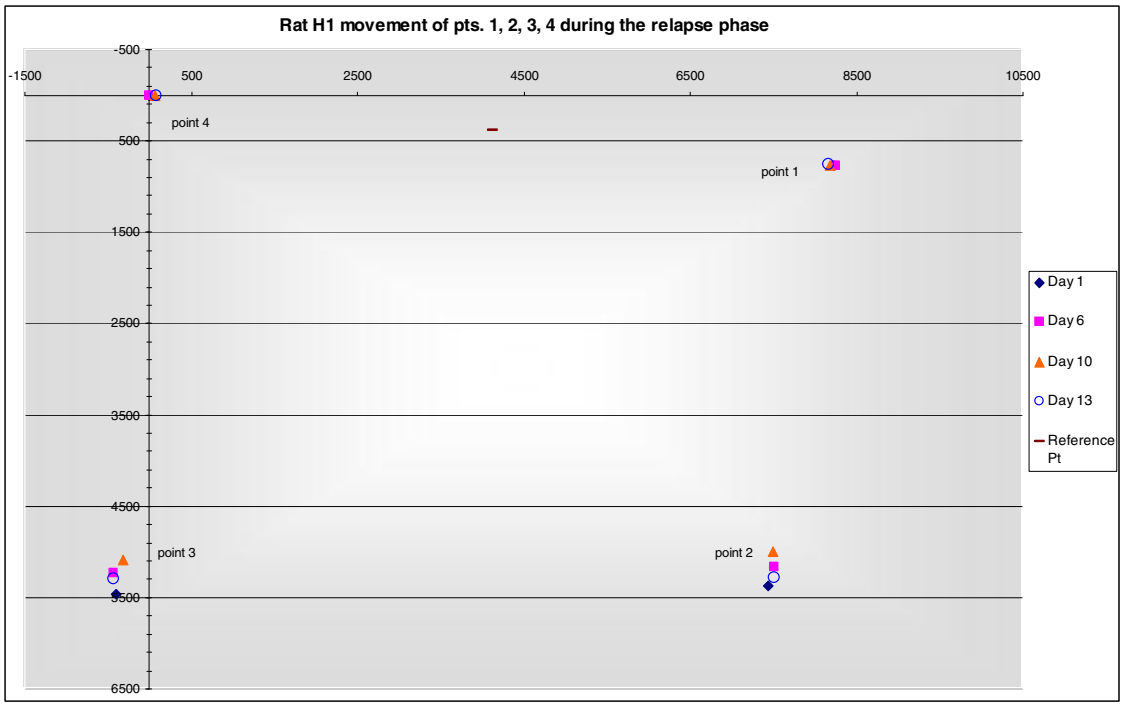

Figure V1: Rat H1 movement of pts. 1, 2, 3, 4 during the relapse phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

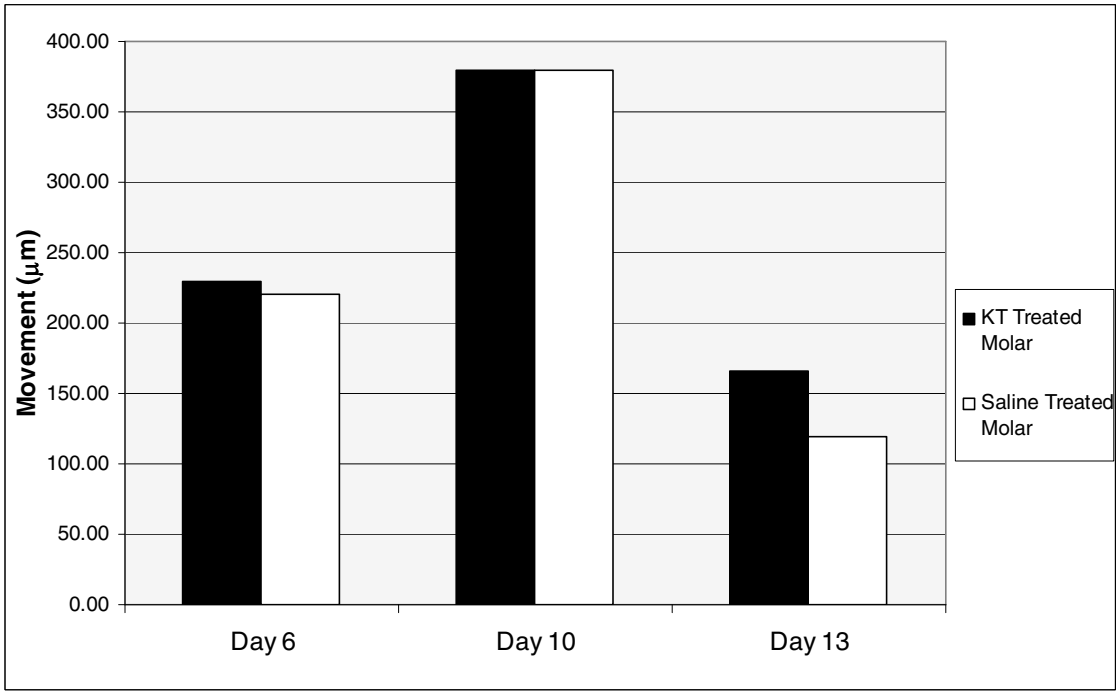

Figure V2: Time-course movements of KT and saline treated first molars during the relapse phase in rat H1. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

APPENDIX W

SUMMARY OF RAT H2 RELAPSE PHASE DATA

Rat H2 tooth movement measurements during relapse phase						
	KT Injection site	**Reference Points**	**Change in X Coordinate (um)**	**Change in Y Coordinate (um)**	**Total movement (um)**	**Relative movement of KT vs. saline treated teeth (%)**
Day 6	UL 1st molar (Pt. 2)	Pt. #2	-33.07	-187.27	190.16	+203.39
		Pt. #3	-13.62	-61.18	62.68	
Day 10	UL 1st molar (Pt. 2)	Pt. #2	-6.90	-215.74	215.85	+43.06
		Pt. #3	111.82	-101.29	150.88	
Day 14	UL 1st molar (Pt. 2)	Pt. #2	39.59	-151.80	156.87	+8.15
		Pt. #3	142.46	-27.33	145.05	

TableW1: Rat H2 tooth movement measurements during relapse phase. Reported in the table are the time points when impressions were taken. In rat H2, KT was injected near the UL first molar (pt. 2), while the UR first molar (pt. 3) was the control side. The total movements of pts. 2 and 3 were measured from the midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1. The percentage difference in movements of the KT treated teeth was calculated relative to the saline treated teeth.

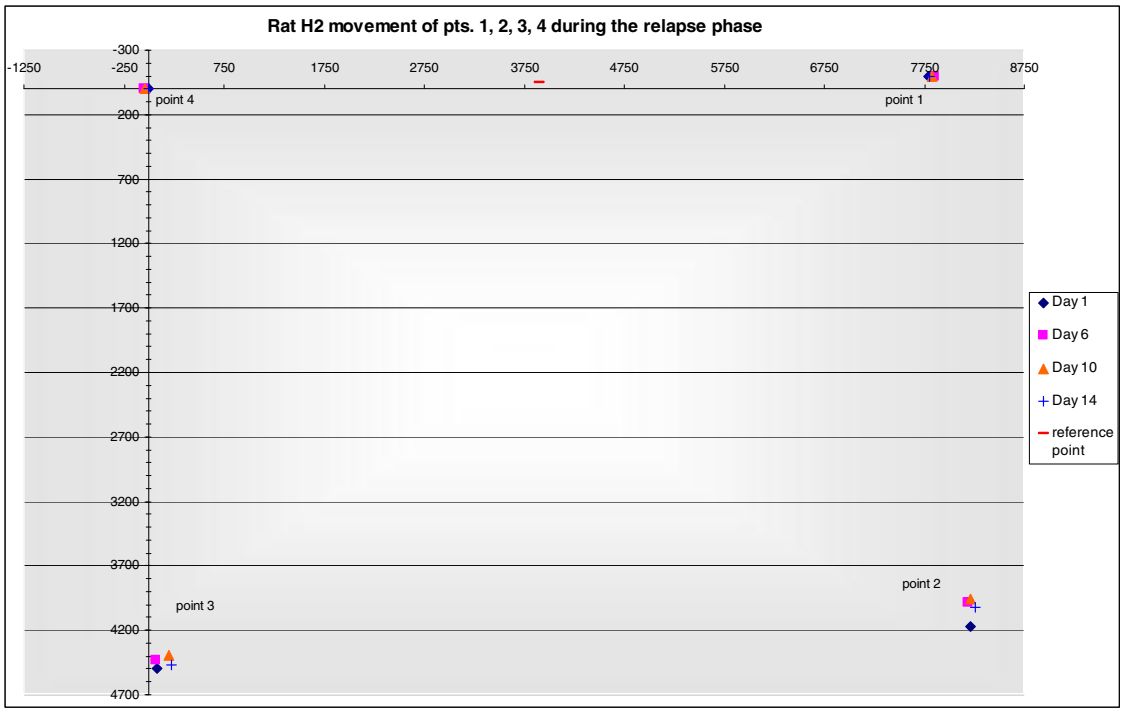

Figure W1: Rat H2 movement of pts. 1, 2, 3, 4 during the relapse phase. Pts. 2 and 3 were localized on the investigative teeth, UL and UR first molars, respectively. Pts. 1 and 4 were localized on the UL and UR third molars, respectively, that did not undergo orthodontic expansion. The midpoint of the line connecting pts. 1 and 4 of the SEM image taken on day 1 was considered the reference point and used to superimpose the consecutive series of SEM images.

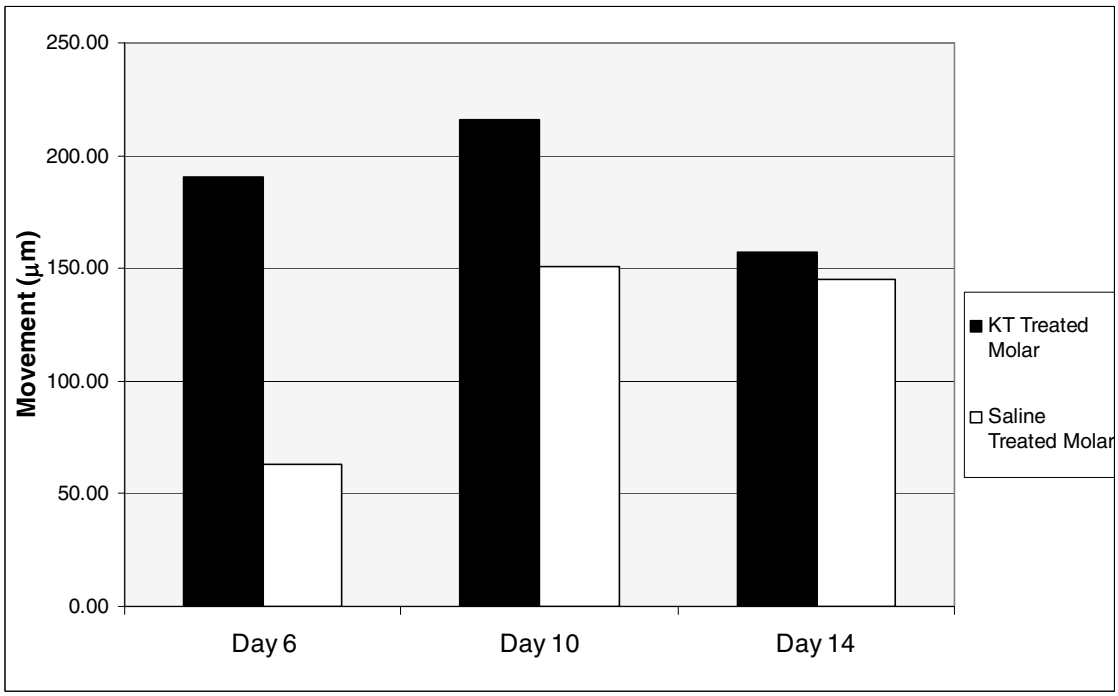

Figure W2 Time-course movements of KT and saline treated first molars during the relapse phase in rat H2. The distances (µm) moved by KT treated teeth (*black bars*) and by the saline treated teeth (*white bars*) are given at each time point.

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