The Effect of Ultrasound on Orthodontic Tooth Movement

by

Dr. Kevin Lloyd Knowlton

A thesis submitted in conformity with the requirements for the degree of Master of Science
Faculty of Dentistry
University of Toronto

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Abstract

Various strategies have been employed to accelerate the rate of orthodontic tooth movement (OTM) in order to decrease treatment time and the associated risks such as periodontal disease and caries. This study investigated whether low-intensity pulsed ultrasound (LIPUS) could accelerate the rate of OTM. Orthodontic patients requiring bilaterally symmetric extraction of first premolars were recruited. During extraction space closure, LIPUS was applied to one side of the dental arch for 20 minutes a day while the contra-lateral side acted as the control. The size of the extraction space was measured throughout treatment. A total of eight patients were considered compliant. The results revealed no significant changes in the rate of OTM with LIPUS during space closure. The findings were contrary to previous studies on dogs and could be related to the dosage or compliance with the coupling gel. Further investigations are needed to determine whether LIPUS application can influence the rate of OTM.
Acknowledgments

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<tr>
<td>AMP</td>
<td>Adenosine Monophosphate</td>
</tr>
<tr>
<td>ARR</td>
<td>Apical Root Resorption</td>
</tr>
<tr>
<td>BMP</td>
<td>Bone Morphogenic Protein</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical User Interface</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LCD</td>
<td>Liquid Crystal Display</td>
</tr>
<tr>
<td>LIPUS</td>
<td>Low-Intensity Pulsed Ultrasound</td>
</tr>
<tr>
<td>NiTi</td>
<td>Nickel Titanium</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>OPG</td>
<td>Osteoprotegrin</td>
</tr>
<tr>
<td>OTM</td>
<td>Orthodontic Tooth Movement</td>
</tr>
<tr>
<td>PDL</td>
<td>Periodontal Ligament</td>
</tr>
<tr>
<td>PGE</td>
<td>Prostaglandin E</td>
</tr>
<tr>
<td>RAP</td>
<td>Regional Accelerated Phenomenon</td>
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<tr>
<td>RANKL</td>
<td>Receptor Activator of Nuclear factor Kappa-B Ligand</td>
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<tr>
<td>SS</td>
<td>Stainless Steel</td>
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<tr>
<td>TNFα</td>
<td>Tumor Necrosis Factor α</td>
</tr>
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<td>USB</td>
<td>Universal Serial Bus</td>
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Chapter 1
Introduction

1.1 Overview

Orthodontic treatment is a process that on average takes 2-3 years to complete. The duration of a traditional orthodontic treatment is limited by the body’s natural ability to remodel the alveolar bone when forces are applied by an orthodontic appliance. From a practitioner’s perspective, minimizing the length of orthodontic treatment is desired in order to prevent or reduce enamel demineralization, gum disease, and loss of patient compliance.\textsuperscript{1-3} The longer the process, the greater the risks. From a patient’s perspective, shorter treatment is preferred due to the physical and visual discomfort associated with braces, along with the missed time from school and work due to added appointments. Advances in treatment efficiency and shorter treatment times can help decrease or eliminate these risks.

This current study is focused on the determining whether or not low-intensity pulsed ultrasound (LIPUS) could increase the rate of tooth movement in patients undergoing orthodontic treatment. The following literature review will focus on four different topics as they relate to the study. It will begin with an overview of the basis for orthodontic tooth movement, followed by a review of the published rates of tooth movement and possible ways to increase this rate. The next area will delve into the application of LIPUS and its use therapeutically to enhance bone remodeling and increase the rate of fracture repair. Finally, the cellular and molecular effects of LIPUS will be discussed along with the potential role of LIPUS in accelerating orthodontic tooth movement.

1.2 Orthodontic Tooth Movement

The classic and most widely recognized theory for orthodontic tooth movement (OTM) is the Pressure-Tension theory. Application of a force to a tooth causes both an area of compression and of tension in the surrounding periodontal ligament (PDL) of the tooth.\textsuperscript{4} Blood flow in the PDL is decreased in the area of compression, whereas it is either maintained or increased in the area of tension. These blood flow changes cause a change in the chemical environment surrounding the tooth. An increase in several different chemical mediators such as cyclic AMP,
nitric oxide (NO), interleukin-1 beta, RANKL, and prostaglandin E (PGE) has been shown in the orthodontic literature.\textsuperscript{5-7} OTM requires the formation of osteoclasts to remove bone adjacent to the compressed portion of the PDL, and osteoblasts to form new bone on the tension side. PGE has been shown to stimulate both osteoclastic and osteoblastic activity and increase the rate of OTM.\textsuperscript{6,8} This concerted effort of osteoclasts resorbing bone, and osteoblasts depositing new bone can also be seen in normal bone growth and remodeling as well as repair of fractures.

A second theory for the biological basis of OTM relies on changes in bone metabolism due to electric signals that are produced when bone is stressed due to flexure and bending. This theory is termed the Piezoelectric or Bioelectric Theory.\textsuperscript{4,9} Piezoelectricity involves the creation of an electric current when a crystal lattice structure is deformed. The current is created as electrons flow from one area of the crystal lattice to another. Since bone is a crystalline structure, it is felt that when orthodontic force is applied to the teeth, there is some bending and deformation of the bone, in part due to the viscoelastic nature of the PDL. In addition, collagen itself is also piezoelectric. These stress signals are important in the maintenance of the bony skeleton. Without these signals, bone tends to atrophy, as seen in the skeletons of astronauts who have been in weightless environments for long periods of time. On top of this, it has been shown that application of an electric field can cause a crystal to deform, and in doing so, create a force.\textsuperscript{10} Experiments have shown that an application of low voltage direct current to alveolar bone can actually cause teeth to move faster.\textsuperscript{11} It is thought that these electric signals affect cell membrane receptors and membrane permeability.\textsuperscript{12}

1.3 Rates of Orthodontic Tooth Movement

In orthodontic practice, the generally accepted rule of thumb is that space closure takes place at a rate of approximately 1mm per month. Several studies have shown this to be fairly accurate.\textsuperscript{13-15} Researchers have come up with several different methods in an attempt to increase this rate of tooth movement. Injections of vitamin D, PGE 1 & 2, osteocalcin, and parathyroid hormone are just a few of the methods that have been used.\textsuperscript{16-20} Although many of these methods have been successful, the invasiveness of the procedures along with the possible systemic side effects makes these methods undesirable for clinical practice. Researchers and product developers have been busy trying to develop less invasive methods of accelerating tooth movement. Currently
there are commercial devices available that use pulsed electromagnetic fields, Light Emitting Diode (LED) lights, and vibrations in an attempt to accelerate orthodontic tooth movement. This study examines another possible device that utilizes ultrasound as a non-invasive method to enhance the rate of tooth movement.

1.4 Low-Intensity Pulsed Ultrasound

Ultrasound refers to sound waves at a frequency of at least 20kHz, which is beyond the range of human hearing (Figure 1). It is a form of mechanical energy that can be transmitted as a high-frequency pressure wave. Ultrasound has many diagnostic or therapeutic applications. Diagnostic ultrasound, also known as medical sonography, utilizes a transducer that both emits ultrasound and records the echoes as the sound waves bounce back. The echoes are used to determine the size, shape, and consistency of tissues and organs. Its use as a diagnostic tool was first described by Dussik in 1942 to locate brain tumors.21 Therapeutic ultrasound uses sound waves to treat tissue injury. Its transducer lacks a recorder for echoes of the sound waves. It is the sound waves themselves that are thought to be therapeutic. The use of therapeutic ultrasound is the basis for this thesis.

The early use of ultrasound was in detecting submarines during World War I.22 It was also at this time that the biological effects of ultrasound were discovered. Fish that were within the ultrasound field experienced violent movements and eventually died. In addition, investigators who put their hand in the field felt severe pain. Therapeutic use of ultrasound using parameters below the pain threshold (frequency of 800 kHz and an intensity of 4-5 W/cm² for 10 min/day for 10 days) was first used in 1939 for treating pain and neuralgias.21,22
Figure 1. Frequency spectrum of sound waves. Acoustic sound waves are audible to humans, whereas infrasound and ultrasound are below and above, respectively, the threshold for human hearing. LIPUS frequency is typically in the 1.5MHz range.

1.4.1 LIPUS parameters and Animal Studies

By 1950, several researchers found that ultrasound had an effect on bone.22,23 The results of these studies were a mixture of positive and negative effects. Some groups found ultrasound to be destructive to bone, while other groups found that, when given below the pain threshold, ultrasound could stimulate bone growth. Comparison of these early studies was difficult due to a lack of established consistent methods for recording therapeutic doses. The initial high intensities that were utilized (500-25000mW/cm²), although capable of stimulating callus formation, ended up eventually causing necrosis of the bone.23 Researchers then began to pulse the ultrasound in order to decrease the overall dosage and subsequent negative effects due to excessive heating of the bone.24

Currently, the commonly accepted LIPUS parameters consist of a 1.5 MHz sine wave repeated at 1 kHz at an intensity (I_SATA) of 30 mW/cm² with a pulse width of 200μs delivered for 20 minutes a day.23 The intensity is low compared to most therapeutic ultrasound parameters so as to decrease heat and thermal effects, as high intensities have been shown to damage bone and delay healing.25,26 Pulsing of the ultrasound decreases the overall intensity, production of heat, and the potential negative effects.24 As the frequency of the ultrasound determines the depth of penetration, the lower the frequency of the ultrasound, the deeper the penetration of the sound waves.27 Therefore, higher frequencies of 3.0 MHz are used for superficial tissue whereas lower frequencies for deeper tissues.27,28 These parameters have been tested and documented through a series of experiments using several different LIPUS treatment regimens and dose rates on animals.
In the first study on the effects of ultrasound on bone healing, Maintz\textsuperscript{21} showed new periosteal bone formation in osteotomized rabbit tibias at low ultrasound intensities. Most current studies have since shown that lower intensities were most effective for fracture treatment whereas higher intensities were found to be less effective. For instance, of the two intensities of either 50 mW/cm\textsuperscript{2} or 100 mW/cm\textsuperscript{2} tested, only the fractured limb of rabbit tibia treated with 50 mW/cm\textsuperscript{2} healed stronger than the control.\textsuperscript{29} Heybeli et al showed that intensities as low as 11.8 mW/cm\textsuperscript{2} improved bone healing.\textsuperscript{30} Xavier and Duarte reported that 70\% of twenty-six nonunions healed after brief exposure (20 min/day) to very low-intensity ultrasound (30 mW/cm\textsuperscript{2}).\textsuperscript{31}

Early studies on LIPUS were conducted on freshly fractured fibulas and femurs with bored holes in rabbits.\textsuperscript{32,33} Using transducers that were available at that time, ultrasound applications with frequencies of 1.65 and 4.93 MHz with intensities of 49.6 and 57 mW/cm\textsuperscript{2}, respectively, were used.\textsuperscript{32} The ultrasound waves were pulsed at 5\textmu s, with a repetition frequency of 1 kHz. No difference was found between the 1.65 and 4.93 MHz frequencies in terms of final outcome of the treated fractures showing more rapid bone growth at the fracture sites, thus allowing for the use of lower frequencies with positive results. This conclusion was also corroborated by the finding of a significant improvement in torsional strength and stiffness of bone only for the 1.5 MHz treated fractures in rats treated with LIPUS at frequencies of 0.5 and 1.5 MHz.\textsuperscript{34} The 1.5 MHz frequency thus appears to have the ideal penetrance, allowing for better bone healing.

In a study on femoral fractures in rats, researchers experimented with pulse widths and repetition rates to examine their effects on bone healing.\textsuperscript{35} They found that a pulse width of 200\textmu s was more effective in enhancing fracture healing as compared to 100 or 400\textmu s. In addition, their results also indicated that a 1 kHz repetition rate was more osteoconductive than a 2 kHz rate.

The time frame over which treatment is given can also change the ultimate healing strength of the bone. Azuma et al applied what could be considered a standard LIPUS dose (30 mW/cm\textsuperscript{2}, 200 \textmu s pulse at 1 KHz with a frequency of 1.0 MHz for 20 min each day) to rat femoral fractures over a course of 24 days in order to assess the time frame over which LIPUS was most effective.\textsuperscript{36} One group received treatment over the full 24 day course, while three other groups received 8 consecutive days of treatment in either the first, second or third week of treatment. There was a statistically significant improved torsional strength and stiffness for all of the groups compared to non-treated controls. However, the 24 day treatment group showed significantly
higher strength and stiffness compared to each of the 8 day groups. Azuma made the suggestion that LIPUS treatment given over time may have an additive effect. Another study used what would be considered the modern standard LIPUS parameters (see below).\textsuperscript{33} From day 17 to 28, all the ultrasound treated fractures had regained an ultimate strength equivalent to intact bone. The non-treated group attained intact strength values only by day 28. LIPUS had accelerated biomechanical healing by a factor of 1.7. No other studies have investigated this phenomenon.

The current accepted parameters for low-intensity pulsed ultrasound appear to be of benefit in bone healing at fracture sites, while eliminating possible negative effects. The lower intensity virtually eliminates any heat production while still being effective, and the low frequency allows for adequate penetration of the tissue. These parameters were used as the basis for human studies.

1.4.2 LIPUS and Fracture Healing in Humans

Throughout the years, several more studies have looked at bone healing with the help of low-intensity pulsed ultrasound (LIPUS) and found that bone healing was accelerated.\textsuperscript{21,32,33} From 1983 to 2014, there have been a number of \textit{in vivo}, \textit{in vitro} and clinical LIPUS studies. A Pubmed search revealed more than 100 articles studying the effect of LIPUS on bone healing and remodeling. Research has shown up to a 40\% improvement in bone healing time for fresh fractures as well as an 85\% improvement in bone healing time in the case of non-unions (based on no additional treatment.).\textsuperscript{23,37-44} It has been suggested that not only does LIPUS improve healing rates but it also reduces the overall medical cost.\textsuperscript{45}

At least four randomized, double-blind, controlled trials performed on humans have concluded that LIPUS has a positive effect on the healing of fresh fractures.\textsuperscript{43,44,46,47} Two of these randomized controlled trials on the effect of LIPUS on fresh fracture healing remain the most referenced human studies.\textsuperscript{43,44} One study examined the effect of LIPUS on tibial fractures, while the other one looked at fractures of the radius. A placebo device was used in the control group. All of the fractures were treated with closed reduction immobilized through the use of a cast that had a window placed in it to allow access for the ultrasound transducers. Healing of the fractures was assessed radiographically at different time points to allow visualization of all four cortices of the bone. The mean time to clinical and radiographic healing was significantly faster in the LIPUS group, with a 38-39\% decrease in healing time. Two other trials found similar
In a study on comminuted tibial shaft fractures, Leung et al. found that LIPUS decreased the time for bone healing and a significantly faster increase in cortical bridging, disappearance of tenderness at the wound site, time to start full weight bearing, and removal of the external fixator and bone mineral content and alkaline phosphatase activity. Research also suggests that LIPUS has a greater effect in people who may have decreased healing potential due to a history of smoking. For example, a 41% and 51% decrease in radiographic and clinical healing time, respectively, were also found in smokers with tibial and radial fresh fractures exposed to LIPS treatment when compared to placebo treated controls. They also reported a 26% and 34% decrease in healing time in non-smokers for radial and tibial fractures.

Not all human studies have found LIPUS to be effective for accelerating bone healing. Three relatively well-controlled double blind clinical trials failed to find a difference in the healing time of fractures exposed to LIPUS. A study on tibial fractures found that the healing time for the LIPUS treated fractures was actually longer than the placebo treated fractures, but the results were not statistically significant. The fractures in this investigation were treated surgically with intramedullary nails, and early weight bearing was encouraged. The stress of the early weight bearing may have limited the effectiveness of the LIPUS. It is also possible that the metallic nail could have attenuated the ultrasound, making it much less effective. Another possible explanation for the lack of effect is that the intramedullary nail allowed for so much stability that the ultrasound could not exert any significant mechanical stresses. Another study assessed fresh fractures of the clavicle that were treated non-operatively. This particular study suffered from lack of objective radiographic findings as their assessment of healing was based solely on clinical criteria such as a resumption of normal activities, pain scales, and the use of pain medications. Furthermore, since ultrasound therapy was given to the experimental group for only 28 days, it might have been difficult to see any added effect from the ultrasound in the short time frame. It was therefore not surprising that no statistical difference in healing times between LIPUS treated fractures and placebo was found. The third study found no observable benefits to using ultrasound as an adjunct to lateral malleolar fractures treated by fixation with bioabsorbable screws. There was no difference in fracture line visualization, callus formation, or bone density throughout 12 weeks of treatment. As no measurements of the times of cortical bridging were taken, an outcome comparison was difficult. The clinical relevance of this study
is also questionable since lateral malleolar fractures also tend to heal rapidly with few complications, so the effect of LIPUS would be minimized.\textsuperscript{51}

A systematic review and meta-analysis on the effects of LIPUS on fracture healing, concluded that LIPUS stimulated bone healing in fresh fractures as judged by radiographic evidence of cortical bridging.\textsuperscript{53} The general conclusion was that the diversity of the studies with respect to the type of bones studied and fixation and immobilization methods used may have affected the effectiveness of LIPUS. A common outcome measure was not present for all studies, so pooling of the data was difficult. Even with these shortcomings, they felt that the evidence for LIPUS in supporting bone healing is substantial. For the treatment of nonunions, the reviewers felt that the evidence in favor of LIPUS was weak. Other literature reviews have been more cautious in their conclusions. Busse et al\textsuperscript{54} stated that the benefits of LIPUS appear to be mainly for fractures treated non-operatively, and that there did not seem to be any added benefit in the presence of fixation with intramedullary nails. Mundi et al\textsuperscript{55} acknowledged that although LIPUS appears to be beneficial to certain fractures, no definitive statement could be made for the universal use in all fractures. LIPUS appears to have the potential to accelerate fracture healing, but more quality studies are needed.

\subsection{1.4.3 Commercial LIPUS Device}

In 1994, the first LIPUS device (Exogen\textsuperscript{®} Bone Healing System, Smith & Nephew Inc., Memphis, TN) was approved by the US FDA for treatment of fractures, and shortly thereafter, was available in Canada.\textsuperscript{21,52,56} Its use was further expanded to the treatment of non-unions in 2000.\textsuperscript{57} The Exogen device was the LIPUS delivery system that was used in the majority of the previously mentioned studies of the effect on bone healing. The device settings for the studies were 1.5 MHz, 30mW/cm\textsuperscript{2}, 200μs pulsed at a 1 kHz repetition rate.

At least 21 studies using the Exogen device have been documented.\textsuperscript{53} The manufacturer claims that fresh fractures have been shown to heal up to 38\% faster, and patients who used the device were able to have their casts removed an average of 22\% sooner than patients with no bone healing device.\textsuperscript{43} In addition, when applied to fractures that have not healed, it has succeeded in healing them in 86\% of cases.\textsuperscript{58} The claim of accelerated fracture healing is based on a randomized controlled trial on tibial fractures.\textsuperscript{43} Radiographic healing of the fractures was significantly faster for LIPUS treated patients versus a placebo control group. The study on the
treatment of non-unions showed that 25 of the initial 29 nonunions of fractures of several different bones that failed to heal within six months of the initial trauma healed.\textsuperscript{58} However, no proper control group with any sort of blinding or placebo treatment was included. In addition, all of the patients had adjunctive therapy prior to the ultrasound application such as bone grafting and other surgical procedures, making it difficult to determine whether the healing was due to the LIPUS or the adjunctive procedures. The manufacturer also claims that the device emits a LIPUS signal which has been shown in laboratory tests to stimulate genes and growth factors that are critical to the body’s natural bone healing process.\textsuperscript{59} Due to the fact that the underlying mechanism of action of LIPUS has not been fully deciphered, the validity of this claim is yet to be determined. Regardless of these claims, because of the popularity and use of the device in the majority of studies and as a commercial therapeutic ultrasound device, the Exogen settings have often been accepted as the LIPUS standard.

1.4.4 Study Device

Smile Sonica Inc. (Edmonton, AB) developed the Aevo System\textsuperscript{TM}, an intraoral LIPUS device that was used in the current study to deliver ultrasound to teeth and the surrounding bone (for more information refer to Appendix A). It is a modification on a device used in an ongoing study on root resorption.\textsuperscript{60} The device consisted of a mouthpiece that was connected to handheld electronics with an LCD that provided information regarding the treatment procedure and status (Figure 2). It is not yet commercially available.
The mouthpiece was to be placed inside the mouth for 20 minutes/day over the entire term of orthodontic space closure. A detailed description of the device is as follows:

**A) Handheld Electronics**

The handheld electronics’ main function was to control the delivery of the LIPUS treatment and to provide information regarding the treatment procedure and status. Information displayed by the handheld electronics included the current state of the device, the remaining treatment time, the battery charge level, and the current date and time. The handheld electronics also maintained a complete record of treatment parameters, including the date and time of each treatment. Access to this information was available through the Aevo System™ GUI. The handheld electronics were powered by a rechargeable lithium polymer battery and were connected to the mouthpiece by the treatment cable. The battery had enough charge to deliver multiple 20 minute treatments. Interaction with the handheld electronics occurred through two buttons on the front panel and information was provided on a LCD. There was also a USB port on the top panel of
the Aevo System™, which was used for charging the battery and connecting to a computer to communicate with the Aevo System™ GUI.

**B) Mouthpiece**

The mouthpiece delivered the LIPUS treatment through the coupling gel and gums to the teeth roots, which were the intended target site. The mouthpiece consisted of a biocompatible material that housed 10 ultrasound emitters and all the internal components of the mouthpiece were hermetically sealed to prevent contact with saliva. The ultrasound emitters were positioned in the mouthpiece to cover both sides of the teeth roots and divide the dental arch into 5 treatment zones, with 5 ultrasound emitters on the buccal (cheek) side and 5 on the lingual (tongue) side. Each treatment zone consisted of two teeth. A model of the mouthpiece and associated treatment zones is shown in Figure 3. Treatment zone 1 corresponded to the left side first and second premolar, treatment zone 2 corresponded to the left side lateral incisor and canine, treatment zone 3 corresponded to the central incisors, treatment zone 4 corresponded to the right side lateral incisor and canine, and treatment zone 5 corresponded to the right side first and second premolar.

![Figure 3. Aevo System™ mouthpiece with treatment zones shown in green.](image-url)
The mouthpiece was similar in structure to a mouthguard and could be inserted into a patient’s mouth in the same fashion. The patient could help keep the mouthpiece in place by biting down on it. With proper positioning of the mouthpiece in the mouth, the ultrasound emitters were positioned over the gums and delivered the LIPUS treatment to the roots of the teeth. The mouthpiece would only be placed inside the mouth once a day for 20 minutes/day over the entire term of the extraction space closure. Separate mouthpieces had been designed to deliver the LIPUS treatment to the different dental arches (either maxilla or mandible). The mouthpiece was provided in one standard size for mandibular arch treatment and one standard size for maxillary arch treatment. The mouthpiece had a flexible structure and accommodated the majority of dental arch shapes and sizes. If treatment of both arches was desired, two mouthpieces could be used at the same time or one after another. A glycerin based ultrasound coupling gel (PDI Inc, Orangeburg, NY) was to be applied to the surface of the buccal and lingual transducers.

**Device Specifications**

The Aevo System™ device used LIPUS in an attempt to accelerate the processes involved in bone remodeling around the teeth roots. The parameters were identical to other LIPUS devices on the market that were used for the treatment of fractures. The 1.5 MHz ultrasound waves were unnoticeable by the patients and the parameters of the ultrasound are summarized in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Value</th>
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<td>Operating frequency (carrier frequency)</td>
<td>1.5 ± 1% MHz</td>
</tr>
<tr>
<td>Amplitude modulation (pulsed)</td>
<td>Repetition rate: 1 ± 1% kHz</td>
</tr>
<tr>
<td></td>
<td>Pulse duration: 200 ± 1% μs ON, 800 ± 1% μs OFF</td>
</tr>
<tr>
<td>Temporal average ultrasound intensity</td>
<td>30 ± 30% mW/cm²</td>
</tr>
</tbody>
</table>

**1.4.5 Mechanism of Action**

The mechanism of action for LIPUS, with respect to bone healing, is not fully understood but is currently being studied. Thermal effects of ultrasound have been noted in the past, but it is not considered to play a role with LIPUS as the intensities currently being used are too low.32,61
Several different explanations for its effects have been suggested, including the possible effects of ultrasound on PGE$_2$ production and action, nitric oxide production, and angiogenesis.

PGE$_2$ comes from the prostaglandin family of derivatives of arachidonic acid via the cyclooxygenase (COX) pathways and is an important regulator of bone remodeling. Studies have shown a bimodal action of PGE$_2$ with respect to bone, with its ability to induce both bone resorption and formation in bone organ cultures, when administered systemically or locally in vivo. PGE$_2$ can stimulate bone resorption by inhibiting osteoprotegrin (OPG) secretion and stimulating RANKL production by osteoblasts leading to an enhanced differentiation of osteoclasts from their precursors. PGE$_2$ has also been shown to increase osteoblast proliferation and differentiation. Studies suggest that the presence of inflammation, dosage, and the presence of different prostaglandin receptors may determine whether bone is resorbed or deposited. Mechanical stimulation of animals, organ cultures, and cell cultures has been demonstrated to be a potent stimulator of PGE$_2$ production and release via osteocytes. LIPUS can be used as a surrogate for mechanical stimulation. In a study on mouse osteoblastic cells, Kokubu et al found an almost three-fold increase in PGE$_2$ production in cells that were exposed to LIPUS. In studies on human osteoblasts, Reher et al not only found an increase in PGE$_2$ production when exposed to ultrasound, but they also found an increase in nitric oxide (NO) production.

NO is a short-lived free radical that is involved in regulating bone turnover and bone cell function. Its production is mediated by nitric oxide synthase (NOS), which can be divided into constitutive (cNOS), and inducible (iNOS) isoforms. The constitutive forms can be found in neuronal (nNOS) and endothelial (eNOS) cells, while iNOS can be found in several cells including osteocytes and osteoclasts. NO has been shown to have an effect on both osteoblast and osteoclast function, and can either stimulate or inhibit bone formation and resorption. The mechanism of action appears to be biphasic, with its effects being dependent on the concentration levels of NO. There has been some confusion in the literature as to the effect of NO concentration on bone metabolism, with some studies claiming a low concentration stimulates bone resorption, while others claim that a high concentration leads to resorption. It appears that the concentration effects are dependent on the NOS isoform responsible for the NO production. In the presence of inflammation, cytokines induce iNOS to release large amounts of NO which have an antiproliferative effect on osteoblasts, increase osteoblast
apoptosis, and enhance osteoclastic bone resorption.\textsuperscript{74-77, 81} This can be seen in osteoporosis where iNOS activation and increased NO contribute to the pathogenesis of the disease.\textsuperscript{77} In a study on rats it was found that orthodontic force lead to an increase in iNOS positive osteocytes on the compression side along with an increase in eNOS positive osteocytes on the tension side of the teeth being moved.\textsuperscript{76} It was postulated that eNOS modulates bone formation while iNOS mediates inflammation induced bone resorption. Several studies have shown that NO is rapidly increased in response to mechanical stress in bone cells,\textsuperscript{82, 83} and LIPUS may simulate this stress.

Angiogenesis is essential to skeletal development and bone remodeling, and vascular endothelial growth factor (VEGF) has an important role in this process.\textsuperscript{84} VEGF has been shown to induce endothelial cell proliferation, migration, and vascular permeability in order to increase blood flow to bone undergoing remodeling.\textsuperscript{85} Adequate circulation to the area undergoing remodeling is important in order to allow bone cells to access the area. In 2004, Wang et al found that exposure to ultrasound significantly increased the amount of VEGF mRNA and protein in cultured human osteoblasts.\textsuperscript{86} They went on further to show that the increase in VEGF was likely mediated by an increase in NO production, which is also a product of ultrasound stimulation. Regardless of how VEGF is increased, LIPUS appears to have the ability to promote angiogenesis.

The possible mechanisms of action for LIPUS continue to be studied. There is still much to be learned, and the picture will likely become much clearer as time goes on.

### 1.5 Potential Role for Low-Intensity Pulsed Ultrasound in Orthodontic Tooth Movement

The effect of ultrasound on the rate of OTM is a relatively new area and there has not been much research in the area, either in animal models or human. Because of the documented effects on PGE\textsubscript{2}, and NO signaling, the following few paragraphs will discuss the indirect evidence for a potential role of LIPUS in OTM.

Studies on the effects of PGE\textsubscript{2} on orthodontic tooth movement have shown an acceleration of movement. Kale et al injected PGE\textsubscript{2} around the teeth of rats that were undergoing distalization. They found that over a 9 day period, the teeth that had the PGE\textsubscript{2} injections moved more than the
controls. In addition, there was a statistically significant increase in osteoclastic activity around the teeth, capillary formation in the bone adjacent to the teeth, and the number of osteoblasts. Other studies on both rats and monkeys have found similar results with respect to accelerated tooth movement. PGE$_2$ is also a well established inflammatory mediator with effects on vascularity, likely through induction of VEGF. An increase in vascularity is essential for the recruitment of cells responsible for remodeling bone and periodontal ligament during OTM.

Several researchers have studied the effects of nitric oxide on orthodontic tooth movement. NO precursors or NOS inhibitors have been used in studies on rats to show that NO can increase the amount of tooth movement over time, therefore reflecting an increase in the rate of tooth movement. Shirazi et al compared groups of rats injected with either L-arginine, a NO precursor, and L-NAME, a NOS inhibitor to a control group of saline injections. Tooth measurements were made throughout the study, and the animals were sacrificed after 13 days for histopathological analysis. The L-arginine group showed a statistically significant increase in tooth movement, while the L-NAME group showed a significant decrease in tooth movement compared to controls. Histopathology demonstrated that the number of osteoclasts was significantly higher in the L-arginine group, while the reverse was true for the L-NAME group compared to the controls. Hayashi et al followed a similar protocol to Shirazi and found the same results. Akin et al only studied NOS inhibitors, and like the others, found that orthodontic tooth movement decreased with a lack of NO production.

Since increases in PGE$_2$, NO, and vascularity are associated with both accelerated OTM, and LIPUS therapy, it is possible that the application of LIPUS may increase the rate of tooth movement.

Safety and efficacy relating to the oral use of LIPUS has been published in the scientific literature. The safety of ultrasonic treatment has been shown in vitro on human gingival fibroblasts. Experiments have shown that the viability of tissue culture cells was not affected, as compared to controls, following 4 weeks of LIPUS treatment. In controlled experiments, LIPUS has been shown to enhance bone formation in osteodistracted rabbit mandibles when applied for 20 minutes per day. It was found that LIPUS accelerated bone formation and maturation when evaluated by photodensitometric, vibratory, elastic, and histological techniques. Experimentation in baboons over a 4 month period was able to capture the
stimulatory effect of LIPUS on their mandible when applied to the temporomandibular joint (TMJ). When compared to the control side, the application of LIPUS to the TMJ increased the mandibular length as well as the growth activity when analyzed with a 99-mTc bone scan. Histological analysis showed that LIPUS increased blood vessel formation and had a positive effect on bone growth, repair, and remodeling. Moreover, a similar LIPUS device has been used in ex-vivo research of a slice organ culture in rats. The study showed that LIPUS enhances alveolar bone remodeling which enhances tooth movement.

In addition, two research studies on dogs were performed at the University of Alberta. In the first study, an ultrasonic dental device prototype significantly accelerated orthodontic tooth movement in dogs over a 4 week period. In a split mouth design on adult beagle dogs (n=10), one side of the mouth was treated with ultrasound for 20 minutes per day and the distance between the third and fourth premolars were measured at the beginning and at the end of the 4-week study. A spring with a constant force was placed between the premolars in order to move them apart. Of the ten dogs, eight dogs showed significant tooth movement acceleration, with an average acceleration of 52%; one dog showed no improvement; and one showed a small deceleration of the treated tooth. A second study involving two adult dogs with the same experimental design as the first study, but for a period of 8 weeks, showed tooth movement accelerations of 45% and 47% as compared with the control. These studies also showed that LIPUS enhanced osteoclastic activity in the alveolar bone and, consequently, enhanced alveolar bone remodeling in dogs. Histological sections showed an increase in the number of osteoclasts on the bone surface adjacent to the PDL in teeth exposed to ultrasound. As a result of these trials, it was postulated that the application of LIPUS had the potential to shorten the duration of the orthodontic treatment by accelerating tooth movement in humans. The authors of the two studies felt that there was a trend towards an increase in tooth movement with LIPUS. Because of the small sample size, statistical significance could not be established; therefore no definitive conclusions could be made. If there was an actual acceleration of tooth movement, then the design of the study may have actually led to an underestimation of the effects. In the study, although the spring was separating two teeth, there were multiple surrounding teeth that would also need to move in order to see an effect. A design in which a tooth was removed and then the teeth on either side of the space were brought together would negate the effect of the
other teeth in the arch, and it would give a purer sense of the effect of the ultrasound as bone remodeling would only have to take place around the two teeth involved.

A clinical trial was conducted on twelve female patients to assess what effect LIPUS had on root resorption during orthodontic treatment. A buccally directed force was applied to bilateral 1st premolars that were destined for extraction as part of their orthodontic treatment plan. LIPUS was applied to the right side of each subject’s mouth while the left side was used as a control. Ultrasound was used for 20 minutes per day for 4 weeks. The device used was the Exogen® Bone Healing System. Exogen ultrasound gel was used to couple the ultrasound energy between the transducer and the mucobuccal fold. No patients experienced discomfort or pain during or after the application of ultrasound. There was no evidence of any deleterious effect on the intervening soft tissues. At the end of the 4 weeks, the 1st premolars were extracted and either underwent study with a scanning electron microscope or histologically. The conclusion reached from this research was that LIPUS minimized root resorption and accelerated healing of the resorption by repairing cementum over 4 weeks of simultaneous tooth movement and LIPUS application. LIPUS treatment during orthodontic treatment in humans has been shown to be a non-invasive method for preventing and minimizing orthodontically-induced tooth root resorption. This characteristic further strengthened the safety profile of LIPUS, relieving concerns that accelerating tooth movement will be unhealthy due to root resorption.

LIPUS has been shown to enhance bone healing and remodeling at various fracture sites throughout the body. Research indicates that part of the mechanism of action involves upregulation of cell signaling molecules such as PGE₂, NO, and vEGF. Orthodontic tooth movement is dependent on the remodeling of bone surrounding the roots of the teeth that have pressure applied to them. The same signaling molecules have been implicated in the biological pathways for OTM. Based on these commonalities, preliminary studies on dogs found that when teeth undergoing OTM were exposed to LIPUS, the rate of tooth movement, on average, was faster than those teeth that were not exposed. To date, no human studies have been undertaken to ascertain whether or not ultrasound therapy can accelerate orthodontic tooth movement.
1.6 Hypothesis

Application of LIPUS during orthodontic tooth movement will allow space closure, following removal of teeth, to proceed at a faster rate than with just orthodontic treatment alone.
Chapter 2
Materials and Methods

2.1 Study Design and Approvals

This study was reviewed and approved for scientific validity and methodology by the Dental Research Institute of the University of Toronto, Faculty of Dentistry. All protocols were approved by the University of Toronto, Research Ethics Board. This study has been registered at www.clinicaltrials.gov. Use of the LIPUS device by the University of Toronto has been approved by Health Canada. All approvals are attached in Appendix A.

Patients undergoing treatment at the University of Toronto, Faculty of Dentistry, Graduate Orthodontics clinic were recruited for the study. All participating patients were previously treatment planned by supervising certified specialists in orthodontics and required bilaterally symmetric extraction of first premolars. The following inclusion and exclusion criteria were applied:

**Inclusion Criteria:**
- Ages 12 to 40
- Orthodontic treatment requiring bilateral extraction of first premolars
- Presence of a minimum of 3mm of extraction space on each side of the arch after initial arch alignment
- Available for follow-up visits
- Willing to sign consent
- Absence of compromising medical conditions
- Good oral hygiene and compliance
Exclusion Criteria:
- Any compromised medical or dental condition that prevented subject from participating in the trial or using the device
- Any implanted assistive devices (e.g. pacemakers, cochlear implants, etc.)
- Currently involved in any other study
- Use of bisphosphonates as they are shown to inhibit orthodontic tooth movement
- Pregnant females

The study design and rationale were verbally explained to potential participants and their parents or guardians. A prototype of the Aevo System™ device was also shown to the potential participants. They were given the opportunity to go over the information included in the informed consent at their leisure and decide whether or not they would participate. Participants were also given the opportunity to ask any questions. Upon signing of the informed consent, participants were monitored during their orthodontic treatment.

In all patients, the following assessment was made at all visits:
1. Compliance with use of LIPUS appliance
2. Amount of extraction space closure – analyzed via measurements on casts
3. Presence of pain or adverse events
4. The degree of root resorption as assessed with radiographs at the first and final visit – Assessment of root resorption was mandated by Health Canada to determine if there was any apical root resorption (ARR) associated with the potential accelerated tooth movement.

2.2 Use of LIPUS

The Aevo System™ LIPUS device and coupling gel were provided by Smile Sonica Inc. at no fee. The device came preprogrammed with one side randomly allocated as the treatment side. The amount of days of treatment needed was programmed by the principal investigator through the use of the Graphical User Interface (GUI) software. When the device was connected to the GUI, the investigator did not have access to see which side of the device was activated.
**Prescription and Instructions**

The participants were given the Aevo System™ device and instructed on how to use the device and how to apply the ultrasound coupling gel. An instruction manual and packages of coupling gel were included in the box for the device. The supplier (Smile Sonica Inc) randomly assigned the devices to have either the right side or left side LIPUS emitters active to coincide with a split-mouth study design. Participants were asked to wear the device for 20 minutes a day until their next visit. They were instructed to fill out a Participant Daily Log for when they used the device, and record any incidents of discomfort on a Pain Reporting Scale. The participants were asked to bring the device with them at each successive visit. The devices were connected to a laptop running the Aevo System™ GUI software. The usage compliance was downloaded from the used device.

Compliance was calculated by dividing the total number of minutes that the device was used during each time period by the total amount of minutes prescribed for the time period (20 minutes/day multiplied by the number of days between time points). A level of 67% was used as the cutoff for inclusion in the study, which was based on the study of a comparable intraoral orthodontic device.¹⁰⁵

### 2.3 Orthodontic Extraction Space Closure

#### Extraction Space Closure Preparation and Mechanics

All orthodontic treatment was undertaken by the Graduate Orthodontic residents originally assigned to each participant. The residents were informed of the study design prior to treatment. The routine orthodontic treatment was not altered for the study.

Preparation for space closure involved initial leveling and aligning with light flexible wires, eventually progressing to stiffer stainless steel (SS) wires. Space closure took place on SS working wires of significant stiffness (round wires of ≥ 0.018” or rectangular wires of ≥ 0.016” x 0.022”) to decrease the likelihood of dental tipping. LIPUS treatment was only initiated once the arches were aligned and ready for space closure.
Sliding mechanics were utilized for the closure of the extraction spaces via distalization of canines. Either full-arch or segmental mechanics were used depending on the case requirements. Nickel-titanium (NiTi) coil springs (GAC, Central Islip, N.Y) of 150g were placed bilaterally from the first molars to the canines to retract the canines into the extraction space. Constant force NiTi springs of 150 grams were chosen as they fall within the range of the generally accepted force for orthodontic tooth movement of 1mm per month.\textsuperscript{13-15} The same type of appliance with the same force magnitude and direction was delivered on both sides of the arch per the split mouth design. In some cases, extra anchorage was added with the use of a Nance or lower lingual holding arch appliance. Anchorage requirements were determined on a case by case basis in order to achieve the desired orthodontic result regardless of the study.

**Orthodontic Appointments and Monitoring of Extraction Space Size and Compliance**

Prior to the initiation of canine retraction, the extraction space remaining after leveling and alignment was assessed. If the extraction space had decreased to less than 3mm prior to active space closure, the participant was excluded from the study even if they had previously signed the informed consent.

The appointment when space closure was to start was considered as baseline and designated T0. Interim visits were undertaken every 4-6 weeks until the extraction space was closed on one side of the arch or it was thought that the space closure would take place prior to the next visit. The initial appointment was labeled as T0 and the first and second interim visits were labeled as T1 and T2, respectively. If the extraction space was not closed after T2, then the following time points were labeled accordingly (T3, T4, etc).

At each of the appointments, dental impressions and a wax bite were obtained. The impressions were taken with stock metal impression trays, using an extended-time irreversible hydrocolloid material (Kromopan). All models were poured by the investigator in yellow stone within 2 hours. The initial extraction space measurements were taken from the stone models. Space closure was monitored, and new dental impressions and a wax bite were taken and dental models were fabricated at each subsequent visit.

Orthodontic visits were scheduled for every 4-6 weeks. At each successive visit, the Aevo System\textsuperscript{™} device was switched with a new device with the emitters activated on the same side as
the original. The used device was then shipped to the supplier for diagnostic tests to confirm that the devices were functioning normally and to inspect for any defects. The Participant Daily Log and Pain Reporting Scale were collected and new ones were given to the participants. The participants were asked if they would like to continue with the study at each visit. The usage compliance was downloaded from the used device. A compliance of 67% was required to continue. If the compliance was below 67% at one visit, it was explained to the participant that more compliance was needed. If at the successive visit the compliance was still below 67%, the participant was asked to withdraw from the study.

**Measurements of Tooth Movement**

Extraction space measurements were performed intra-orally, and on all of the dental models by the investigator. The extraction space measurements were taken with a digital caliper (Samona, 0-150mm, resolution 0.01mm) at the height of contour interproximally between the canines and second premolars. A daily rate of tooth movement was calculated by dividing the millimetric change in interproximal space by the total number of treatment days. The rate was multiplied by 28 days to calculate a monthly rate of tooth movement that could be compared to previous orthodontic tooth movement studies.

Following the completion of the study, a second Graduate Orthodontic resident also made extraction space measurements on ten random study models. Both were blinded as to which side of the arch received the LIPUS treatment. A second set of measurements was made on the same ten random models by the principal investigator in order to allow for both inter and intra-operator reliability.

**Root Resorption Analysis**

At the final visit, periapical radiographs of the retracted canines were taken, along with the standard dental impressions and wax bite registration. The radiographs were taken with plain films that were positioned using a RINN holder. The films were scanned and imported into Adobe Photoshop. To correct for angulation and magnification differences when the radiographs were taken, the height of the orthodontic brackets was measured clinically and on the radiographs to use as a reference in order to calculate more accurate tooth lengths.
2.4 Data and Statistical Analysis

The following data was collected for each participant:

- Age at onset of space closure
- Sex
- Size of extraction space at each time point (every 4-6 weeks) until space on one size closed
- Wire size during space closure
- Side of dental arch receiving the LIPUS therapy
- Compliance rate for each time period and overall
- Number of days participant was in the study
- Root lengths of each canine prior to, and at the end of the study

Statistical analysis was performed by a statistician using IBM SPSS Statistics for Windows version 22. Paired t-tests were utilized for both the rates of extraction space closure and the changes in root length. A P value of ≤ 0.05 was considered to be significant. Intraclass coefficients were calculated for inter-rater and intra-rater reliability.
Chapter 3
Results

3.1 Patient Sample

The initial recruitment of participants was from the 2012 intake of patients at the University of Toronto Graduate Orthodontic clinic. From this cohort, patients who required bilateral extractions of 1st premolars as part of their treatment plan were asked to volunteer for the study. A total of 10 patients initially consented to be part of the study. These 10 patients underwent extractions, some initial alignment of their teeth, and leveling of their curve of Spee prior to initiation of space closure of the extraction spaces. After initial alignment, 2 of the 10 recruits were not able to continue to the experimental stage of the study due to the presence of extraction spaces that were less than 3mm. Five more out of the remaining were excluded due to a lack of device compliance (compliance rates ranged from 0% to 26%). The remaining 3 participants had device compliance rates of 79.3%, 81.3%, and 94.7%. Due to the low sample size, a second round of recruitment of the 2013 intake of patients to the Graduate Orthodontic clinic was undertaken. Thirteen new patients with a total of 14 dental arches (both mandibular and maxillary arches from one patient were included) consented to participate in the study. Of these participants, 4 did not start treatment due to delays in having teeth extracted, delinquency in attending their regular orthodontic appointments, or complications with alignment of their arches. Another 5 participants were excluded due to a lack of compliance (3% to 35%). In total, over both rounds of recruitment, 7 participant arches met the compliance criteria of 67%. Another participant with a compliance of 63.8% was deemed to be borderline, and was included in the sample for statistical analysis. A total of 8 dental arches were included in the final sample. Table 2 outlines the descriptive statistics of the study population.
Table 2. Descriptive statistics for the study sample.

<table>
<thead>
<tr>
<th>Age</th>
<th>16.89 (+/- 2.30) years (range 11.2-18.9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>2 males, 6 females</td>
</tr>
<tr>
<td>Dental Arch</td>
<td>4 mandible, 4 maxilla</td>
</tr>
<tr>
<td>LIPUS activated side</td>
<td>6 right, 2 left</td>
</tr>
<tr>
<td>T0-T1</td>
<td>30.00 days (range 28-41)</td>
</tr>
<tr>
<td>T1-T2</td>
<td>28.13 days (range 21-36)</td>
</tr>
<tr>
<td>Compliance (%)</td>
<td>82.18 (range 63.8-96.3)</td>
</tr>
</tbody>
</table>

3.2 Experimental Time Frames

For 7 of the 8 completed arches, the space closure was measured at two time points beyond the initial baseline measurement (T0-T1, T1-T2; Table 2). The first time period consisted of the time between the start of treatment (T0) and the first orthodontic adjustment visit (T1). The average duration of the first time period was 30.00 days with a range of 28-41 days. The average duration of T1 and the second (T2) orthodontic adjustment visits was 28.13 days with a range of 21-36 days. For one of the arches, two extra time points were measured, with the consent of the patient, as the extraction space was not closed at T2. The two extra time periods of this patient had durations of 36 and 35 days.

3.3 LIPUS Device Compliance

Compliance was recorded by the user through the use of a written daily log and by the device itself. When there was a discrepancy between the participants’ written daily log, and the downloaded device log, the device log was used for the study as it was considered to be more accurate. A calculation of compliance (based on the total number of minutes of device use/time
period divided by total number of the prescribed 20 minutes use) gave an average rate of 82.18% with a range of 63.8%-96.3% for those considered compliant. The compliance rate for the non-compliers ranged from 0% to 35.3%.

### 3.4 Space Closure Measurements

Measurements of the extraction spaces from dental casts were taken at T0, and the following two appliance adjustment appointments (T1 and T2). Ten models from the dental casts of 8 treated arches taken at different time points were selected at random, and the spaces on both the left and right sides were measured again by both the principal investigator and another orthodontic resident (JS). A total of 20 measurements were then compared in order to assess intra-rater and inter-rater reliability. The intraclass correlation coefficient (ICC) in each case was >0.99, therefore showing a high degree of consistency. The initial set of dental cast measurements was also compared to the intra-oral measurements, which also resulted in an ICC >0.99. This high degree of consistency also allowed for the use of the initial dental cast measurements for the statistical analysis.

![Figure 4](image)

**Figure 4.** Extraction space in LIPUS and control sides within the same subjects at baseline. The diagonal reference line is plotted where Y=X.
The average starting extraction space at T0 on the treatment side was slightly larger than space on the control side (0.3 +/- 0.9; Figure 4) but the differences were not statistically significant (paired t-test: P=0.33). In addition, the extraction space at T1 on the treatment side was still slightly larger (0.4 +/- 0.9; P=0.44).

### 3.5 Rates of Extraction Space Closure

#### a) Differences in space change between treatment and control teeth

Comparison of the rate of space closure (changes in size of extraction space from T0-T1 and T1-T2) revealed that the overall average rate of space closure was 1.2 mm/month on the LIPUS side compared to a 1.3mm/month on the control side (Table 3). Paired t-tests showed that difference was not statistically significant between the sides for all measurements and time points. A percent reduction in extraction space was also calculated in order to take into account the difference in the initial extraction space sizes at T0. This also revealed that the difference between the two sides, with respect to the rate of space closure, was not statistically significant. Although the average rate of space closure was similar between the two sides, there were variations between the subjects with some exhibiting a faster rate of tooth movement on the LIPUS side while others have the control side move faster (Table 4).

A monthly rate of space closure was calculated with the inclusion of the extra time points for the one participant still revealed a lack of significant difference between LIPUS treated teeth and controls (P=0.43). Calculation of the rate of OTM by excluding the participant with the borderline compliance rate of below 67% threshold (63.8%) also revealed a non-significant difference between treated and control teeth (P=0.66). Additionally, a paired t-test was performed with the non-compliers included (who used the device more than once) and the difference was also non-significant (P=0.99).
Table 3. Mean and standard deviation (in brackets) of measurements of space change in ultrasound and control teeth at T1 and T2.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Time point</th>
<th>LIPUS Side</th>
<th>Control Side</th>
<th>Paired t-test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction in space (mm) from baseline</td>
<td>T1</td>
<td>1.5 (0.5)</td>
<td>1.6 (0.5)</td>
<td>0.14 (0.6)</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>2.4 (0.4)</td>
<td>2.6 (0.7)</td>
<td>0.19 (0.7)</td>
</tr>
<tr>
<td>Total percent reduction in space from baseline</td>
<td>T1</td>
<td>31% (14)</td>
<td>36% (12)</td>
<td>4% (14)</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>50% (14)</td>
<td>57% (18)</td>
<td>7% (16)</td>
</tr>
<tr>
<td>Monthly percent reduction in space from baseline</td>
<td>T1</td>
<td>32% (15)</td>
<td>36% (13)</td>
<td>4% (15)</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>26% (8)</td>
<td>30% (9)</td>
<td>3% (8)</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>29% (11)</td>
<td>33% (10)</td>
<td>4% (11)</td>
</tr>
<tr>
<td>Monthly rate of space closure (mm/month)</td>
<td>Overall</td>
<td>1.2 (0.2)</td>
<td>1.3 (0.3)</td>
<td>0.2 (0.2)</td>
</tr>
</tbody>
</table>

Table 4. Overall monthly rate of space closure (T0-T2) and device compliance for all compliant participants including the borderline complier.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Rate of Space Closure (mm/month)</th>
<th>Device Compliance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LIPUS</td>
<td>Control</td>
</tr>
<tr>
<td>001</td>
<td>0.91</td>
<td>1.56</td>
</tr>
<tr>
<td>008</td>
<td>1.48</td>
<td>1.52</td>
</tr>
<tr>
<td>009</td>
<td>1.09</td>
<td>0.70</td>
</tr>
<tr>
<td>011</td>
<td>1.01</td>
<td>1.32</td>
</tr>
<tr>
<td>016</td>
<td>1.00</td>
<td>1.20</td>
</tr>
<tr>
<td>017</td>
<td>1.27</td>
<td>1.27</td>
</tr>
<tr>
<td>018</td>
<td>1.54</td>
<td>1.44</td>
</tr>
<tr>
<td>024</td>
<td>1.03</td>
<td>0.98</td>
</tr>
<tr>
<td>Mean</td>
<td>1.16</td>
<td>1.25</td>
</tr>
</tbody>
</table>
b) Differences between mandible and maxilla and males and females

There was an average percent change of 34.5% (±10%) for the four participants who had treatment in the mandible, and 31.3% (±12%) for the four patients who had treatment in the maxilla. The differences were not statistically significant (P=0.67). Since there were only two male patients, a statistical analysis to compare any gender differences in the rate of tooth movement was not performed.

3.6 Difference in Root Length Change

Root length changes of the retracted canines as measured using periapical radiographs at T0 and the end of LIPUS treatment showed that the root length for both the control and ultrasound treated teeth decreased by about 2% over time (Figure 6). The resulting t-tests analyzing both millimetric and percent reductions in tooth length were statistically insignificant (Table 5).

![Figure 5](image-url)

**Figure 5.** Boxplots showing the change in root length of control and ultrasound teeth.
Table 5. Mean and standard deviation (in brackets) of change in root length of ultrasound and control teeth between T0 and final measurement (approximately 60 days). Also shown are the results of paired t-tests comparing ultrasound and control teeth within individual subjects.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Ultrasound tooth</th>
<th>Control tooth</th>
<th>Paired t-test results (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction in root length (mm)</td>
<td>-0.59 (0.28)</td>
<td>-0.60 (0.33)</td>
<td>0.89</td>
</tr>
<tr>
<td>% Reduction in root length</td>
<td>2% (1.0)</td>
<td>2% (1.0)</td>
<td>0.47</td>
</tr>
<tr>
<td>% Reduction in root length per month</td>
<td>1% (0.6)</td>
<td>1% (0.5)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

3.7 Pain or Adverse Events

One of the non-compliers complained of the mouthpiece digging into her gingiva. She ended up only wearing the device one day and subsequently dropped out of the study. No other participants complained of any pain or adverse events.

3.8 Summary of Results

The study failed to find a statistically significant difference in the rate of extraction space closure between LIPUS treated teeth and non-LIPUS treated teeth. In addition, there was no difference in the amount of root resorption detected. The data indicates that both the initial extraction space sizes and initial tooth lengths were similar for both the treatment group and controls. No correlations with arch location were detected. Based on the data from this current study, the hypothesis that the application of LIPUS during orthodontic tooth movement will allow space closure to proceed at a faster rate than with just orthodontics alone, is rejected.
Chapter 4
Discussion

This study failed to show a significant difference in the orthodontic tooth movement rates of teeth exposed to low-intensity pulsed ultrasound, therefore leading to a rejection of the hypothesis. The results differed from previous studies performed on dogs. The following discussion outlines some of the possible reasons for the lack of effect as well as touching on some issues with the study design and the utilization of a split-mouth method.

4.1 Sample Size

A sample size calculation prior to the study (Appendix B) found that 10 subjects were required for a power of 80% with a significance of 0.05. Two years of recruitment and treatment resulted in a sample size of eight. Ideally another round of recruitment to obtain a sample size greater than 10 should have taken place. Due to time constraints, an additional round of recruitment could not take place. One could argue that the study is underpowered and therefore the results cannot be deemed definitive. In spite of a small sample size, however, the statistical analysis did in fact show fairly convincing results with the P values for the paired t-tests not reaching statistical significance. The study is also currently being undertaken at the Universities of Alberta and Manitoba. The results may be pooled after the completion of all the studies to come up with a greater sample size. The results of this study will not be revealed to the other centers or the public until the other studies have been completed.

4.2 Compliance

One of the problems that was encountered in this current study was the relatively lack of compliance with respect to using the ultrasound device as prescribed. It is difficult to complete most forms of orthodontic treatment without some sort of patient compliance requirement. Whether it involves using elastic bands, wearing a functional appliance or headgear, wearing a retainer, or even maintaining good oral hygiene, motivating a patient to assist with treatment can be a difficult task. The same can be said for the Aevo System™ LIPUS mouthpiece that was
used in the study. Of the 18 participants who were given the device to use, only 7 of them met the 67% compliance outlined in the protocol (8 if the borderline participant at 63.8% was included). The participants were only asked to wear the device for 20 minutes a day, which is far less than the time typically required for headgear or elastic wear. Because of the lack of compliance, the minimum sample size was not met, therefore reducing the power of the study.

No form of reward was offered to the participants other than the possibility of treatment progressing faster on one side of the arch. One could argue that the use of a reward would have helped increase compliance and therefore increase the sample size. That claim, however, has been refuted. Richter et al. looked at the effect of rewards on patient motivation for two groups: above average compliers, and below average compliers. They found that the above average compliers remained above average throughout treatment after a reward was given as motivation. The below average compliers improved once a reward was given, but never reached the compliance levels achieved by the above average compliers. With that said, it is doubtful that a reward would have had enough of an effect on the poor compliers in the current study in order to get them to the 67% cut-off. As mentioned before, when the non-compliers were included in the t-test, the difference in the rate of OTM between the sides was non-significant.

An added level of compliance was involved in the study with respect to the use of the ultrasound coupling gel. Not only did the participants have to wear the Aevo System™ device, they also had to apply the gel each time they used the device. A demonstration on how to apply the gel and how much to use was given at the initial delivery of the device. A bag of single use packages of gel were given to the participants at each visit. There were more than enough packages to cover the amount of days between visits. In some cases during subsequent visits, there were more packages remaining in the bag than what would have been expected based on the number of days of appliance usage. In these cases, instructions on the use of the gel were given again, and the importance of the gel was stressed. Although the gel was innocuous and tasteless, some of the participants had negative feedback. The lack of taste and the gel consistency were said to be unpleasant and the participants did not look forward to using the gel. Considering the complaints about the gel, and the excess remaining gel packages, there is reason to question whether the participants actually used the gel as directed. If it was not used properly, then this could explain the lack of effect of the ultrasound. The air/tissue interface provides a highly reflective boundary which may bounce back up to 99% of the incident ultrasound.
energy.\textsuperscript{107} The use of the coupling gel eliminates the reflection of the ultrasound. Even the presence of small air pockets can reflect a significant amount of the energy. If the participants were not using the gel, or were not using enough of it, then the ultrasound was not reaching the tissue in order to have an effect.

Previous studies on dogs exhibited positive findings when teeth undergoing OTM were treated with LIPUS. It is important to point out the main difference between those studies and the current one. The compliance rate for the dogs was 100% as the ultrasound was applied by the researchers with the coupling gel and transducer directly applied to the area. In contrast, the average compliance rate in the current study was 82.18% with a range of 63.8%–96.3%. These values in patients were obtained based not only on the subject wearing the device but without any guarantee that the coupling gel was applied correctly and that the ultrasound penetrated to the targeted teeth and bone.

4.3 Split-Mouth Study Design

The first recorded use of the split-mouth design was in 1968 by Ramfjord et al in a trial comparing two types of periodontal therapy.\textsuperscript{108} The design has the benefit of not only allowing between-patient comparisons, as in most studies, but also within-patient comparisons. The use of each participant as their own control eliminates the need to recruit a control group that matches the treatment group in such areas as age, sex, and extraction space size. By using participants as their own control, a smaller sample size could be utilized. Based on the sample size calculation for the study, a minimum of 10 participants and 10 controls were required for a total of 20 participants. Because of the split-mouth design, a total of only 10 participants were needed because they acted as their own controls. This is an added benefit because of the difficulty in recruiting participants for the study due to the strict inclusion criteria.

A split-mouth reduces the possibility of any confounding variable having an effect on the outcomes. An assumption can be made that biological factors such as bone metabolism, bone density, and canine root length are consistent between the two sides of the mouth. In addition, the same appliance and wire type and dimension was used for both the control and treatment sides. Reducing these extraneous factors increases the probability that the difference between
the treatment and control sides with respect to the rate of orthodontic tooth movement is due to the actual ultrasound treatment. In this case, the rate of tooth movement was not significantly different between the two sides, so it can be said with greater certainty that LIPUS does not appear to have an effect on OTM.

There are two drawbacks to the split-mouth design that may or may not have had an effect on the outcome: leakage of LIPUS to the control side, and the carry-across effect of circulating chemical mediators.

**Leakage of LIPUS from the Treatment to Control Side**

The design of the Aevo System™ mouthpiece consists of five ultrasound transducers on both the lingual and buccal sides of the mouth (total of ten transducers). For the study, the treatment side had four transducers activated at the canine and bicuspid regions whereas the anterior transducers associated with the incisor teeth were not activated. This reduced the risk of possible leakage of ultrasound energy from the treatment side of the mouth to the control side. Direct measurements and computer simulations were performed by the device manufacturer to confirm that the control side of the arch did not receive ultrasonic treatment through accidental coupling of the ultrasound waves from the active side. These tests measured negligible ultrasound intensity levels (<5%, or <1.5 mW/cm²) coupled between the two sides. Such low power levels are not known to cause any biological reaction in tissue. Based on that, it can be assumed that no significant amount of ultrasound would have reached the control side of the dental arch. Therefore, leakage of the ultrasound would not explain the lack of difference in the rate of OTM between the experimental and control teeth.

**The Carry-Across Effect**

The carry-across effect happens when a local treatment has systemic effects. It is conceivable that if the LIPUS treatment did in fact increase PGE₂ and NO production, then these mediators could also travel through the circulation to the control side of the mouth and induce an effect at sites distant from the area of treatment. This would lead to an underestimation of the effect of the ultrasound therapy, or in the worst case, would show no difference between the two sides. Since there is no anatomical barrier in the maxilla and mandible to prevent this from happening, carry-across is plausible, but is unlikely. Prostaglandins are hormone-like substances, but unlike
most endocrine hormones that travel through the bloodstream to distant sites to exert their action, they are locally acting. They can be classified as autocrine or paracrine as they act either on the same cell that secretes the prostaglandin, or on nearby cells.\textsuperscript{110,111} In addition, prostaglandins undergo rapid metabolic degradation. Their ability to act at distant sites is minimal to non-existent. NO is a highly reactive free radical that is also considered to be a paracrine mediator, so its effects are localized to the cells adjacent to where it was secreted.\textsuperscript{112} Due to its high reactivity, the ability of NO to reach distant sites is limited.

In orthopedic surgery, a remarkable amount of remodeling activity occurs adjacent to the site of injury. Frost first described this reaction as a regional accelerated phenomenon (RAP).\textsuperscript{113,114} It consists of a transient burst of localized remodeling process following surgical injury to the bone. It is suggested that osteoclasts and osteoblasts do not exist in sufficient numbers to heal the bone after surgery, so more of these cells are recruited to the area.\textsuperscript{113-115} This phenomenon is used to explain why teeth appear to move faster after orthognathic surgery is performed.\textsuperscript{116} It is also the basis for accelerated osteogenic orthodontics in which corticotomies in the alveolar bone surrounding teeth causes an increased rate of OTM.\textsuperscript{117-119} Bone remodeling at one site can appear to have an effect on the remodeling at adjacent anatomical sites. If this were the case, then it is feasible that ultrasound treatment could not only lead to bone remodeling at the site of activation, but also at distant sites such as the other side of the dental arch. In 2009, Mostafa et al actually negated this possibility of a carry-across effect in a split-mouth designed study.\textsuperscript{120} In their study, bilateral maxillary second premolars were removed in dogs, and miniscrews were placed to facilitate the distalization of the first premolars with the use of NiTi coils. On one side of the mouth, corticotomies were performed to help facilitate OTM, while the other side consisted of standard tooth movement techniques without corticotomies. They found the rate of tooth movement on the corticotomy-assisted side to be almost double that of the standard side. The RAP caused by the corticotomy did not appear to crossover to the other side of the dental arch, or at least it did not have a significant effect. Several other split-mouth studies have also exhibited results that downplay the possibility of a carry-across effect. Research using the split-mouth design to study the effects of low-level laser therapy (LLLTT),\textsuperscript{13,121-124} pulsed electromagnetic fields,\textsuperscript{125} and local injections of prostaglandins\textsuperscript{20,87,126} all found a significant increase in the rate of tooth movement on the treatment side of the mouth as compared to the controls. If there was a carry-across effect in these studies, it did not dampen the perceived
effects to the point that no difference was found. Each of the therapies maintained a more localized effect. The previous studies on dogs exposed to LIPUS also resulted in a significant difference in the rates of tooth movement.\textsuperscript{101,103} So, much like the other methods of accelerating tooth movement, LIPUS also would appear to have a more localized effect.

If the carry-across effect was so significant that it accelerated the OTM on both sides of the mouth equally, then one would expect the rates of tooth movement on each side to be higher than the recorded norms. As part of the sample size calculation for this study, several articles were reviewed in an attempt to find an average rate of orthodontic space closure (Appendix A). When combined, these studies exhibited an average monthly tooth movement rate of 1.11 mm per month with a range of 0.60 - 1.70 mm/month. The monthly rates of 1.25 mm/month and 1.16 mm/month for the control and LIPUS sides of the mouth, respectively, both fall within the range of what would be considered normal. This helps validate a lack of systemic effect. The control side actually had a slightly higher rate of tooth movement, which would mean the carry-across would be more effective than direct ultrasound exposure. This is highly unlikely. It is also unlikely that LIPUS has the effect of slowing down OTM as the rate differences were not considered statistically different.

\section{4.4 Other Factors}

The LIPUS parameters used in the study are typical of those applied externally to bone fractures. Successful healing in most fracture studies has been determined by bridging of the cortical bone. The typical LIPUS settings appear to have a positive effect on cortical bone. In OTM, the bone that is undergoing remodeling is not cortical. The bone adjacent to the PDL is less dense than that of the outer cortex, and it is surrounded by abundant trabecular bone. It may be possible that the current settings do not have the same positive effect on trabecular bone. It may be that the operating frequency needs to be decreased (< 1.5 MHz) in order to penetrate through the outer cortex of the alveolus to act upon the PDL and surrounding bone.

The 150g force used for retraction of the canine has been used in several other split-mouth designed studies on tooth movement. 150g is in the upper range of the optimum force for bodily movement of a single tooth via frontal resorption.\textsuperscript{4} Heavy forces have been shown to decrease
the rate of tooth movement due excess compression of the PDL and the formation of a “hyalinized” or necrotic and avascular area of bone on the leading edge of the tooth. These leads to a delay in movement as osteoclasts need to attack the underside of the lamina dura through undermining resorption in order for tooth movement to take place. It can take anywhere from 7-14 days for tooth movement to occur through undermining resorption. One could argue that the 150g force used in the study was excessive, and that the resulting hyalinization lead to a delay in tooth movement, therefore affecting the rate in both the LIPUS treated and control teeth. If this were the case, then the rates of OTM from T0-T1 could be identical. However, by T1, the effect of hyalinization would no longer be present. A discernible difference in the rate of tooth movement between the two sides should have been observed from T1-T2 if LIPUS treatment had a positive effect on OTM. The results do not reflect this. In fact, the control side extraction space closed slightly faster during this time period, but the difference was non-significant.

4.5 Root Length Measurements

Periapical radiographs of the retracted canines were used to monitor the amount of apical root resorption (ARR). There was a concern that if the application of ultrasound caused an increase in the rate of OTM, then it was possible that ARR may also be more significant. One study found a significant reduction in ARR was found in LIPUS exposed premolar teeth that were tipped buccally prior to their extraction. The biomechanical set-up in our current study involved translation of the roots of the teeth, a movement that required more force, therefore posing a greater risk of ARR. Because of this risk, it was deemed wise to monitor the canine root lengths.

Periapical radiographs historically have been used to diagnose ARR. They do however have their drawbacks. Since teeth are tipped and rotated during orthodontic movement, it is difficult to achieve identical irradiation geometry for the initial and final radiographs with this technique. Radiographs taken at different angles can lead to lengthening or shortening of the radiographic projection of the tooth. To overcome this, the heights of the orthodontic brackets were measured clinically and radiographically, and the resulting distortion was quantified and applied to the tooth length measurements. The overall accuracy of this technique
is questionable, but it was felt to be adequate for this study as we were only interested in detecting root resorption of larger and more clinically relevant magnitudes. CBCT has been shown to be more accurate than periapical radiographs for detecting root resorption, but the added radiation exposure was not deemed to be warranted in this study.129

The changes in tooth length over the course of the study were virtually the same for the control (0.60mm) and LIPUS (0.59mm) exposed teeth (P=0.94). This was to be expected as the rate of extraction space closure also showed no significant difference between the two sides. Since the LIPUS exposed teeth did not have an accelerated rate of tooth movement, then there would be no reason for the root resorption to increase. The lack of a significant difference in root resorption also suggests that LIPUS exposure in this study did not reduce the severity of ARR found previously.104

4.6 Future Directions

Similar studies are currently being undertaken at the University of Alberta and the University of Manitoba. Due to the low sample size in this study, it is likely that the results of this study will be pooled with the results from the other centres in order to achieve more significant results.

4.7 Conclusions

The results of this study suggest that the application of low-intensity pulsed ultrasound via an intra-oral mouthpiece does not affect the rate of orthodontic tooth movement associated with extraction space closure. As with any removable orthodontic device, a lack of compliance appears to be an issue. The study was underpowered due to a small sample size, however the findings were still rather convincing. Further study with a larger sample size is recommended in order to verify the results. Development of a more pleasant coupling gel is also recommended.
5.1 Abstract for poster presentation at the University of Toronto, Faculty of Dentistry Research Day February 2014

The Effect of Ultrasound on Orthodontic Tooth Movement

K KNOWLTON*, B TOMPSON, S-G GONG, A METAXAS

Department of Orthodontics, Faculty of Dentistry, University of Toronto

**Background:** Shorter orthodontic treatment time can help alleviate many of the risks of treatment such as enamel decalcification, caries, and periodontal disease. Various strategies have been employed to accelerate the rate of orthodontic tooth movement (OTM). A possible strategy includes the use of low-intensity pulsed ultrasound (LIPUS), a non-invasive and safe treatment modality. One of the proposed mechanisms of action is an increase in PGE$_2$ and NO production, both of which have been implicated in orthodontic tooth movement.

**Objective:** To study the effect of LIPUS on the rate of orthodontic space closure in humans.

**Methods:** Patients requiring bilateral extraction of first premolars as part of their orthodontic treatment were recruited from the University of Toronto Graduate Orthodontic Clinic. Canine retraction was utilized to close the extraction space. A LIPUS device (Aevo System™, Smile Sonica Inc) designed to fit over teeth and braces was given to each participant to use at home 20 minutes a day until the extraction space closed on one side of the mouth. The study was double-blind, with the LIPUS emitters only active on one side of the mouth. Measurements were made on dental casts in order to calculate the rate of tooth movement for each side of the mouth. The compliance rate was downloaded from the device at each visit.

**Results:** A lack of compliance led to several drop-outs. Three participants from the initial pool of volunteers successfully completed the study. Treatment took an average of 54 days. There were varying rates of tooth movement, with differences of 15-42% between sides. The study is currently ongoing, and the investigator is still blind as to which side received the LIPUS. Participant recruitment will continue until an adequate number of participants have completed the study.

**Grants:** University of Toronto, Faculty of Dentistry Research Committee Grant.
References


60. El-Bialy, T. and University of Alberta Graduate Orthodontics Program, clinical study (ClinicalTrials.gov identifier: NCT00423956).


Appendix A

A1. Scientific Merit Approval from the Dental Research Institute, Faculty of Dentistry – University of Toronto

Faculty of Dentistry
University of Toronto

Dr. B. Ganss
Vice-Associate Dean (Research)
Phone: (416) 416-978-8728
Email: b.ganss@utoronto.ca

Ref. # 12-13-9

January 9, 2013

Dr. Bryan Tompson
Faculty of Dentistry
University of Toronto

Dear Dr. Tompson,

Thank you for your responses regarding your application entitled “The effect of low-intensity pulsed ultrasound (LIPUS) on orthodontic tooth movement.” Your project is now approved for scientific merit and funding for the amount of $1200.

Please read the attached Faculty grant guidelines carefully and address any issues directly with Ms. Leah Raz. Leah will set up the account for your research project and provide you with the appropriate instructions for accessing the funds.

I wish to also remind you that in accordance with the grant policy, you must submit a one page project report, which will also include as an attachment, a list of any documents produced by the applicants or trainee(s) (i.e. Research Committee presentations, abstracts, papers, thesis, etc.), and/or submitted funding applications to external agencies, that were directly related to the Dental Faculty Research Grant (DFRG) funding. The report is to be submitted within 6 months following one year from the date of funding allocation (i.e. 18 months following receipt of funding).

Any unspent funds remaining in the account after one year from the funding date will be returned to the research fund pool. If you will require an extension on the work for reasons that were beyond your control you must make your request in writing, addressed to the Associate Dean Research, c/o Leah Raz, Dental Research Institute, prior to 1 month of the termination date for your grant.

I wish you all the best in this study and I am looking forward to seeing the results at the Faculty’s Annual Research Day.

Sincerely,

Leah Raz for
Dr. Bernhard Ganss
Vice-Associate Dean (Research)

cc: Dr. Siew-Ging Gong
Dr. Kevin Krowlton
124 Edward Street  Toronto Ontario  M5G 1G6  FAX (416) 979-4770
A2. Ethics Approval from the Research Ethics Board – University of Toronto

UNIVERSITY OF TORONTO

PROTOCOL REFERENCE # 28380

January 8, 2013

Dr. Bryan Tompson
FACULTY OF DENTISTRY

Dr. Kevin Knowlton
FACULTY OF DENTISTRY

Dear Dr. Tompson and Dr. Kevin Knowlton,

Re: Your research protocol entitled, "The effect of low-intensity pulsed ultrasound (LIPUS) on orthodontic tooth movement"

ETHICS APPROVAL

Original Approval Date: January 8, 2013
Expiry Date: January 7, 2014
Continuing Review Level: 1

We are writing to advise you that the Health Sciences Research Ethics Board (REB) has granted approval to the above-named research protocol under the REB’s delegated review process. Your protocol has been approved for a period of one year and ongoing research under this protocol must be renewed prior to the expiry date.

Any changes to the approved protocol or consent materials must be reviewed and approved through the amendment process prior to its implementation. Any adverse or unanticipated events in the research should be reported to the Office of Research Ethics as soon as possible.

Please ensure that you submit an Annual Renewal Form or a Study Completion Report 15 to 30 days prior to the expiry date of your current ethics approval. Note that annual renewals for studies cannot be accepted more than 30 days prior to the date of expiry.

If your research is funded by a third party, please contact the assigned Research Funding Officer in Research Services to ensure that your funds are released.

Best wishes for the successful completion of your research.

Yours sincerely,

Judith Friedland, Ph.D.
REB Chair

Daniel Gyewu
REB Manager

OFFICE OF RESEARCH ETHICS
McMurchy Building, 12 Queen's Park Crescent West, 2nd Floor, Toronto, ON M5S 1S8 Canada
Tel: +1 416 946-3573 • Fax: +1 416 946-5763 • ethics.review@utoronto.ca • http://www.research.utoronto.ca/for-researchers-administrators/ethics/
PROTOCOL REFERENCE # 28380

December 19, 2013

Dr. Bryan Tompson

FACULTY OF DENTISTRY

Dr. Kevin Knowlton

FACULTY OF DENTISTRY

Dear Dr. Tompson and Dr. Kevin Knowlton,

Re: Your research protocol entitled, "The effect of low-intensity pulsed ultrasound (LIPUS) on orthodontic tooth movement"

<table>
<thead>
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<th>ETHICS APPROVAL</th>
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</tr>
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<tr>
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<td>Expiry Date: January 7, 2015</td>
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<td></td>
<td>Continuing Review Level: 1</td>
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<tr>
<td></td>
<td>Renewal: 2 of 4</td>
</tr>
</tbody>
</table>

We are writing to advise you that you have been granted annual renewal of ethics approval to the above-referenced research protocol through the Research Ethics Board (REB) delegated process. Please note that all protocols involving ongoing data collection or interaction with human participants are subject to re-evaluation after 5 years. Ongoing research under this protocol must be renewed prior to the expiry date.

Please ensure that you submit an Annual Renewal Form or a Study Completion Report 15 to 30 days prior to the expiry date of your protocol. Note that annual renewals for protocols cannot be accepted more than 30 days prior to the date of expiry as per our guidelines.

Any changes to the approved protocol or consent materials must be reviewed and approved through the amendment process prior to its implementation. Any adverse or unanticipated events should be reported to the Office of Research Ethics as soon as possible. If your research is funded by a third party, please contact the assigned Research Funding Officer in Research Services to ensure that your funds are released.

Best wishes for the successful completion of your research.

Yours sincerely,

[Signature]

Elizabeth Peter, Ph.D.
REB Chair

[Signature]

Daniel Gyewu
REB Manager
A3. Health Canada Approval for Investigational Testing of Aevo System™

04/02/2013 10:38 FAX 613 957 9969 MBB DED 

Therapeutic Products Directorate
2934 Baseline Road, Tower B
Address Locator: 3403A
Ottawa, ON K1A 0K9

DATE: APR 02 2013
Application No. 197850

John Simon
President/Owner
John Simon & Associates Ltd.
1416-115 Street NW
Edmonton AB T6J 7B8

Investigational Testing Authorization – Revised - Class III

Dear John Simon:

This is in reference to your Authorization to conduct Investigational Testing in Canada issued on 9 October 2012, and your request to amend the study protocol and recruit an additional investigator received on 28 March 2013 and submitted pursuant to Part 3 of the Medical Devices Regulations. This pertains to the following:

Protocol: The Effect of Ultrasound on Orthodontic Tooth Movement

Number: PR-0018, Rev 2
PR-0031

Date: 2 February 2013
5 February 2013

Objectives: To study whether or not the Aevo System™ medical device enhances the rate of tooth movement in human subjects who use orthodontic braces.

Device: Aevo System

No. of devices: Fifty (50)

No. of subjects: Fifty (50)

The information has been reviewed and you are hereby authorized to amend the study protocol and add Dr. Kevin Knowlton, DDS and Co-Investigator Dr. Bryan Tompson, DDS, FRCDS(C) to the list of investigator(s). This Authorization for Investigational Testing supersedes the Authorization issued on 9 October 2012.

Canada

Imdb-brun@RegulationsPart3AuthoritiesforInvestigationalTestingAuthoritiesbyManufacturerNameSmileorion Inc197150-IT Rev Auth lett.doc
Sections 86, 87 and 88 of the Medical Devices Regulations impose additional requirements regarding the advertisement, record keeping and labelling of devices involved in investigational trials. Please advise the Bureau of any changes to the device, protocol or list of investigators. Any changes to the device or protocol that fall outside the scope of the risk assessment of this protocol will require a new application.

Yours sincerely,

Roland Potter, Ph.D.
Director
Medical Devices Bureau

RR/sw
Attach.
A4. Participant Informed Consent Form, Daily Log, and Pain Reporting Form

PARTICIPANT CONSENT FORM

Title: The Effect of Ultrasound on Orthodontic Tooth Movement

Principal Investigator: Dr. Kevin Knowlton Ph: 416-979-4750 ext 3037

Why am I being asked to take part in this research study?

You have been asked to take part in this research study because you need to have teeth removed as part of your orthodontic treatment and the extraction space to be closed using orthodontic braces.

What is the reason for doing the study?

One of the main challenges is to reduce the length of the orthodontic treatment without causing bad effects, such as pain or tooth root shortening. Shortening treatment time prevents or reduces loss of tooth enamel and gum problems. Shorter treatment may be better because of the discomfort from wearing braces.

Wearing braces could cause teeth roots to shorten. Ultrasound devices speed up bone healing and possibly prevent root shortening.

This research study will test the use of the Aevo System™, a low-intensity pulsed ultrasound (LIPUS) dental device, along with braces, to speed up bone healing and tooth movement. This treatment may result in a shorter time wearing orthodontic braces.

What will I be asked to do?

Your complete orthodontic treatment will be given to you in the Graduate Orthodontic Clinic at the University of Toronto. The expected length of the study is 24 weeks or until the extraction space (the space between your teeth) is closed on either side to the satisfaction of Dr. Knowlton. The time may vary, more or less, depending on how fast your teeth move. Your complete orthodontic treatment may last 24 months.

Taking part in the study will require up to 7 visits to the clinic. Each visit will last about 15 min to 1 hour. These visits will be part of your normal orthodontic treatment, and no extra visits are required.
**Week 0 Procedures:**

After your teeth extraction and some initial aligning of your teeth, you will be randomly assigned (like flipping a coin) to receive ultrasound treatment to one side of your mouth along with your braces.

You will have your tooth measurements taken, a root length x-ray done, and dental impressions (teeth molds) taken. Orthodontic appliances for gap closing of the teeth will be attached to the teeth on both sides of your mouth.

You will be shown how to use the ultrasound device and will take it home with you. You will use the device for twenty minutes each day for up to 24 weeks. The ultrasound device will provide ultrasound treatment to half of your teeth and placebo treatment (no ultrasound) to the other half of your teeth. Neither you nor the study doctor will know which part of the device is giving the ultrasound.

We will give you a form to fill out if you feel any discomfort. You can rate the discomfort on a scale from 1 to 10, from “No Pain” to “Worst Pain”. You will need to write the exact date and time the pain occurs on the form and what, if anything that you do about it. There should be no discomfort from using the ultrasound device.

You will also be given a daily log (a paper sheet) to write down the times that you wear the ultrasound device and any changes that you notice between the two sides of your mouth.

You will need to bring the ultrasound device with you to each clinic visit. The study doctor will download usage data and update the treatment for the next 4 week period. The device will be inspected for damage. The study doctor may give you a different device to allow inspection of your device by the study sponsor to ensure all parts of the device are working properly.

You will be instructed about medications or food supplements that you should not use. Some medications affect the rate of tooth movement. Certain pain medication will be prescribed by the study doctor and no other pain medication should be taken.

**Weeks 4, 8, 12, 16, 20 Procedures:**

You will come to the clinic to check the appliance and your tooth measurements for any changes. Your orthodontic appliance will be adjusted as required for usual orthodontic care. Dental impressions will be taken.

**Week 24 Procedures:**

You will come to the clinic for your final visit. Tooth measurements will be taken. Your orthodontic appliance will be adjusted as usual care. Dental impressions will be taken and root length x-rays will be done again.
If the space between your teeth closes sooner than 24 weeks, the Week 24 procedures will be completed at that time and your part in the study will end. After the study, you will continue regular orthodontic treatment.

**What are the benefits to me?**

Taking part in this study will not change the quality your treatment. A potential benefit would be that your teeth may move faster to close the extraction spaces, however, you may not get any benefit from being in this research study. The information collected from this study could help us speed up orthodontic tooth movement and reduce the length of time patients need to wear braces in the future.

**What are the risks and discomforts?**

There is no expected risk to you outside of the risks involved with normal orthodontic treatment. Because the ultrasound is safe for humans, there should be no risk to teeth. It is important, however, that you immediately tell the study doctor if you have any unusual symptoms or have any concerns. It is not possible to know all of the risks that may happen in a study.

If you take part in this research, you will be exposed to a very small amount of radiation from dental x-rays. These x-rays are in addition to the normal x-rays that you will receive during your orthodontic treatment. They are taken in order to monitor the health of the roots of the teeth that we are moving.

**Will my information be kept private?**

Personal records relating to this study will be kept confidential. Any research data collected about you during this study will not identify you by name. You will be assigned a coded number for the length of this research study. Your name will not be disclosed outside the research clinic. Any report published as a result of this study will not identify you by name.

The study doctor may need to look at your personal health records held at the University of Toronto Faculty Of Dentistry and/or kept by other health care providers that you may have seen in the past (i.e. your family doctor). Any personal information we get from these records will be only what is needed for the study.

During research studies it is important that the data we get is accurate. For this reason your health data, including your name, may be looked at by people from Health Canada, the Health Research Ethics Board, and SmileSonica’s delegated monitor and quality assurance.

By signing this consent form you are saying it is okay for the study doctor/staff to collect, use and disclose information about you from your personal health records as described above.
After the study is done, we will still need to securely store your health data that was collected as part of the study. The sponsor requires the data from this study to be stored for 25 years.

If you leave the study, we will not collect new health information about you, but we will need to keep the data that we have already collected.

Do I have to take part in the study?

Being in this study is your choice. If you decide to be in the study, you can change your mind and stop being in the study at any time, and it will in no way affect the care or treatment to which you are entitled.

Can my participation in the study end early?

You are free to withdraw from the research study at any time, and your future orthodontic care will not be affected in any way. If you withdraw from the study, all data collected up to the date of withdrawal will be kept but no new information will be collected about you.

Are there other choices to being in this research study?

If you choose not to be in this study, you will receive regular orthodontic treatment without an ultrasound device.

What will it cost me to participate?

There is no cost to you for participation in this study.

Will I be paid to be in the research?

You will not be paid for your participation in this study.

What if I have questions?

If you have any questions or concerns about the study, you may contact Dr. Kevin Knowlton by phone at 416-979-4750 ext 3037.

If you have any questions regarding your rights as a research participant, you may contact the Office of Research Ethics at 416-946-3273. This office has no affiliation with the study investigators.
PARTICIPANT CONSENT FORM

Title: The Effect of Ultrasound on Orthodontic Tooth Movement
Principal Investigator: Dr. Kevin Knowlton Ph: 416-979-4750 ext 3037

Do you understand that you have been asked to be in a research study? ☐ ☐

Have you read and received a copy of the attached Information Sheet? ☐ ☐

Do you understand the benefits and risks involved in taking part in this research study? ☐ ☐

Have you had an opportunity to ask questions and discuss this study? ☐ ☐

Do you understand that you are free to refuse to participate or withdraw from the research study at any time? This will not affect the results of your orthodontic treatment. ☐ ☐

Do you understand who will have access to your records, including personally identifiable health information? ☐ ☐

Do you want the investigator(s) to inform your family doctor that you are participating in this research study? If so, give his/her name ____________________________

Who explained this study to you? _______________________________________

I agree to take part in this study: YES ☐ NO ☐

__________________________________________________________
Participant’s Signature Printed Name Date

I agree that my child may take part in this research study.

__________________________________________________________
Parent’s Signature Printed Name Date

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

__________________________________________________________
Signature of Investigator Or Designee Printed Name Date

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH PARTICIPANT
The Effect of Ultrasound on Orthodontic Tooth Movement - Participant Daily Log
Participant Initials ___ ___ ___     ID #_______

Record the times that you wear the device and any changes that you notice between the two sides (left and right) of your mouth.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time Device Put On</th>
<th>Time Device Taken Off</th>
<th>Changes between sides</th>
<th>Activity while wearing the device</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No ☐ Yes ☑</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>If Yes, specify:</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>No ☐ Yes ☑</td>
<td></td>
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<td></td>
<td>If Yes, specify:</td>
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<td>No ☐ Yes ☑</td>
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<td>If Yes, specify:</td>
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<td>No ☐ Yes ☑</td>
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<td>No ☐ Yes ☑</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>If Yes, specify:</td>
<td></td>
</tr>
</tbody>
</table>

Version 1, 22 Jun 12
The Effect of Ultrasound on Orthodontic Tooth Movement - Participant Pain Reporting Scale

Subject Initials: ___ ___ ___      ID #:________________

If you have pain or discomfort using the LIPUS, please complete the pain scale. Bring this form to your next visit.

Date: 
Time: 

Indicate source of Pain: 
1. Braces
2. Ultrasound device
   a. While wearing
   b. After removal

Check the box in the table below that corresponds to the amount of pain you feel (1 = no pain and 10 = worst pain).

<table>
<thead>
<tr>
<th>Location of pain</th>
<th>No Pain</th>
<th>Worst Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top arch - Left side</td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td>Top arch - Right side</td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td>Bottom arch - Left side</td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td>Bottom arch - Right Side</td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
</tbody>
</table>

Write down if you do anything to treat the pain.

Pain Treatment:

Aevo System™
Investigational Device:
To be used only under the care of a
Qualified Investigator

Instructions for Use
Date: 20 December 2012
Status: Final

CAUTION: Federal Law restricts this device to sale, distribution or use by or on the order of a medical professional. Use is restricted to the individual for whom it is prescribed.
1. Indications for Use
The Aivo System™ is indicated for shortening the duration of orthodontic treatment. It accelerates alveolar bone remodeling resulting in faster tooth movement, while preventing orthodontically-induced tooth root resorption and reducing pain due to orthodontic braces.

2. Contraindications
There are no known contraindications.

3. General Warnings
Use as directed. Do not exceed 20 minutes/day.

4. Precautions
The effect of the Aivo System™ on active implantable devices (e.g., cardiac pacemakers, hearing aids, etc.) has not been evaluated. The operation of active implantable devices may be adversely affected by close exposure to the Aivo System™. Research subjects using an active implantable device are recommended to be evaluated by their attending cardiologist or physician.

In a previous clinical study, research subjects were instructed to apply a device similar to the Aivo System™ for 20 minutes/day. The safety and effectiveness of the Aivo System™ when used for any treatment duration other than 20 minutes/day is unknown.

5. Adverse Effects
Laboratory [1], animal [2, 3], and clinical research studies [4] for intra-oral dental applications using the same ultrasound parameters as the Aivo System™ were reported in literature with no significant adverse effects or medical complications.

6. Abbreviations
The abbreviations used in this document are summarized in Table 1.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>GUI</td>
<td>Graphical User Interface</td>
</tr>
<tr>
<td>LIURS</td>
<td>Low-Intensity Pulsed Ultrasound</td>
</tr>
</tbody>
</table>
7. Device Description

The Aevo System™ provides orthosonic™ treatment, which shortens the duration of orthodontic treatment by accelerating tooth movement and alveolar bone remodeling with low-intensity pulsed ultrasound (LIPUS). The Aevo System™ is battery powered, portable, intended for home use, and is used as an add-on to traditional orthodontic treatment, complementing orthodontic braces (e.g. wire braces, clear aligners).

The Aevo System™, as shown in Figure 1, consists of a mouthpiece that is connected to handheld electronics with an LCD that provides information regarding the treatment procedure and status. For the present clinical trial, one side of the mouthpiece will be active and one side will be inactive (placebo). The mouthpiece will only be placed inside the research subject’s mouth once a day for the 20 minute treatment for the duration of the clinical trial.

The Aevo System™ will be used by a single research subject and will be returned to the Principal Investigator at the end of the treatment. The Principal Investigator will instruct the research subject on the correct usage of the device and the research subject will apply the daily treatment at home.

6. Ultrasound Technical Specifications

The Aevo System™ ultrasound parameters are shown in Table 2. Neither the Principal Investigator nor the research subject can select or change any of the Aevo System™ ultrasound parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating frequency (carrier frequency)</td>
<td>1.6 ± 0.1 MHz</td>
</tr>
<tr>
<td>Modulating signal burst width</td>
<td>200 ± 50 μs</td>
</tr>
<tr>
<td>Repetition rate</td>
<td>1.0 ± 0.1 kHz</td>
</tr>
<tr>
<td>Spatial average-temporal average (SATA)</td>
<td>30 ± 30% mW/cm²</td>
</tr>
<tr>
<td>Spatial average-temporal maximum (SATM)</td>
<td>150 ± 300 mW/cm²</td>
</tr>
</tbody>
</table>

9. Device Components

9.1 Handheld Electronics

The handheld electronics’ main function is to control the delivery of the orthosonic™ treatment and to provide information regarding the treatment procedure and status. Information displayed by the handheld electronics includes the current state of the device, the remaining treatment time, the battery charge level, and the current date and time. The handheld electronics also maintain a complete record of the date and time of each treatment, which is available to the Principal Investigator through the Aevo System™ graphic user interface (GUI).

The handheld electronics are powered by a rechargeable lithium battery and are connected to the mouthpiece by the treatment cable. The battery has enough charge to deliver multiple 20 minute treatments. Interaction with the handheld electronics occurs through two buttons on the front panel and information is provided on an LCD. There is also a USB port on the top panel of the Aevo System™, which is used for charging the battery and connecting to a computer to communicate with the Aevo System™ GUI. Figure 2 shows the front and back panel view of the handheld electronics, while Figure 3 shows top and bottom panel views.
9.2 Mouthpiece

The mouthpiece, as shown in Figure 4, delivers the orthosonic™ treatment through the coupling gel and gams to the teeth roots, which are the intended treatment site. The mouthpiece consists of a biocompatible material that houses 10 ultrasound emitters and all the internal components of the mouthpiece are hermetically sealed to prevent contact with saliva. The ultrasound emitters are positioned in the mouthpiece to cover both sides of the teeth roots and divide the dental arch into 5 treatment zones, with 5 ultrasound emitters on the buccal (cheek) side and 5 on the lingual (tongue) side. Each treatment zone consists of two teeth. A model of the mouthpiece and associated treatment zones is shown in Figure 5.

The mouthpiece is similar in structure to a mouthguard and should be inserted in the research subject’s mouth in the same fashion. The research subject has to keep the mouthpiece in place by biting down on it. With proper positioning of the mouthpiece in the research subject’s mouth, the ultrasound emitters are positioned over the gums and can deliver the orthosonic™ treatment to the teeth roots. The mouthpiece will only be placed inside the research subject’s mouth once a day for the 20 minute treatment for the duration of the clinical trial.

Separate mouthpieces have been designed to deliver the orthosonic™ treatment to the different dental arches (either maxilla or mandible). The mouthpiece is provided in one standard size for mandible arch treatment and one standard size for maxilla arch treatment. The mouthpiece has a flexible structure and accommodates the majority of dental arch shapes and sizes. The Principal Investigator will recommend whether the mandible or maxilla mouthpiece should be used by the research subject.

For the purposes of this clinical trial, either the right (treatment zones 1 and 2) or left (treatment zones 4 and 5) side of the mouthpiece will have active ultrasound emitters and the other side will have inactive (placebo) ultrasound emitters. Both the active and inactive sides of the mouthpiece are identical in appearance and the emitted ultrasound is unnoticeable by the research subject. Each Aero System™ provided for the trial will have the active side of the mouthpiece pre-selected by the device manufacturer, and neither the Principal Investigator nor the research subject will be told which side is active.

Figure 4: Aero System™ mouthpiece.

9.3 Treatment Cable

The treatment cable has medical grade insulation and connects the mouthpiece to the handheld electronics. It is a permanent part of the Aero System™ and cannot be disconnected. Should the treatment cable become damaged or disconnected, the Aero System™ will need to be returned to the Principal Investigator.

9.4 Coupling Gel

Ultrasound coupling gel will be provided for use during each treatment. The coupling gel is to be applied to the inside of the mouthpiece before the start of each treatment so that the orthosonic™ treatment can be properly transmitted from the mouthpiece through the gums to the teeth roots. The coupling gel should only be applied to the mouthpiece and not to any other parts of the Aero System™.

Only a thin layer (approximately 3 to 4 mm thick, or about 0.5 mL in volume) of coupling gel should be applied to the inside of the mouthpiece. Just enough to ensure contact between the mouthpiece and the research subject’s gums when the mouthpiece is placed in the mouth. The coupling gel should be applied along the ultrasound emitters on the buccal and lingual sides of the mouthpiece, as highlighted in Figure 5. Care should be taken to avoid swallowing the coupling gel and the research subject should rinse out his or her mouth with water following each treatment.

The research subject should only use the coupling gel provided with the Aero System™. If any adverse reaction to the coupling gel occurs, please contact the Principal Investigator. Other coupling gels were not designed or tested for use with the Aero System™.

Figure 5: Aero System™ mouthpiece with treatment zones highlighted. The treatment zone numbers are imprinted on the exterior of every mouthpiece.

Treatment zone 1 corresponds to the right side first and second premolar
Treatment zone 2 corresponds to the right side lateral incisor and canine
Treatment zone 3 corresponds to the central incisors
Treatment zone 4 corresponds to the left side lateral incisor and canine
Treatment zone 5 corresponds to the left side first and second premolar.
9.5 Charger

The charger is a wall mount power adapter with a USB port that can be connected to the handheld electronics with the USB cable. The charger allows the Aero System™ battery to be recharged when there is insufficient charge to provide a treatment. The charger is intended for use with the Aero System™ only. Additionally, as a safety feature, the treatment cannot be activated when the Aero System™ is connected to the charger and the charger is plugged into an electrical outlet.

9.6 USB Cable

The USB cable can be used to connect the handheld electronics to the charger to recharge the Aero System™ battery. Additionally, the USB cable can be used to connect the handheld electronics to a computer to communicate with the Aero System™ GUI.

10. Device Operating Instructions

10.1 Normal Operation

1. Prepare for treatment. Prior to using the Aero System™:
   - Ensure you are in a location where you will be comfortable for the 20 minute treatment duration.
   - Ensure that your mouth is clean and free from any food particles or other items that may interfere with the treatment.

2. Inspect the Aero System™. Visually check the mouthpiece and treatment cable for any cracks or signs of damage prior to use. If there are any cracks or damage, do not use the device and please return it to the Principal Investigator.

3. Apply coupling gel to the mouthpiece. Apply a thin layer (approximately 3 to 4 mm thick, or about 3 mL in volume) of coupling gel to the inner surface of the mouthpiece, along the ultrasound emitters on the buccal and lingual sides.

   Note: Whenever the mouthpiece is removed from your mouth, it should be cleaned and reinserted and a new application of coupling gel is required before reinserting the mouthpiece in your mouth.

4. Place the mouthpiece in your mouth. The mouthpiece should be inserted into your mouth and over your teeth the same as you would with a mouthguard. The ultrasound emitters should be positioned over the gums, as described by the Principal Investigator during your initial visit.

5. Turn on the Aero System™. Press the button to turn on the Aero System™. The SmileSonic logo will be displayed briefly, followed by instructions to begin the treatment.

6. Start treatment. Press the button to begin a 20 minute treatment. A countdown timer will display the remaining treatment time.

7. Pause treatment. The treatment can be paused at any point during the treatment cycle by pressing the button.

8. Treatment finished. Once the countdown timer reaches 0:00, the Aero System™ automatically stops the treatment and displays a message that the treatment is complete. Remove the mouthpiece from your mouth, spit out any excess coupling gel, and rinse your mouth with water.

9. Turn off the Aero System™. Hold the button for at least 2 seconds to turn off the Aero System™.

10. Connect and store the Aero System™ for next use. Using a clean, damp, soft cloth or paper towel, wipe off any excess coupling gel from the mouthpiece. Clean the mouthpiece with a manual toothbrush and toothpaste, using light pressure. Rinse the mouthpiece with cold tap water. Let the mouthpiece dry at room temperature and store it as you would any household electronic device. Ensure the Aero System™ is turned off prior to storage.

10.2 Charging the Battery

1. Low battery. The Aero System™ will not deliver the full 20 minute treatment unless the battery has sufficient charge. A message will be displayed when there is insufficient battery life remaining to deliver treatment.

2. Remove the mouthpiece from your mouth. The mouthpiece should be removed from your mouth before charging the battery.

3. Inspect the charger. Visually check the charger and USB cable for any cracks or signs of damage prior to use. If there are any cracks or damage, do not use the charger and please return it to the Principal Investigator.

4. Connect the charger to the Aero System™. Insert the USB cable into the USB port on the top panel of the handheld electronics and plug the other end into the charger.

5. Begin charging the battery. Plug the charger into an electrical outlet to begin charging. While the battery is charging, all treatment will be stopped.

6. Charging finished. The Aero System™ will display a message when charging is complete. If the battery was empty, charging may take up to 6 hours to complete.

7. Disconnect the charger from the Aero System™. Disconnect the charger from the handheld electronics and resume normal operation of the Aero System™.

10.3 Error Condition

If any error occurs, reset the device by turning the device off and turning it back on again. If the error persists, please contact the Principal Investigator.
11. GUI Operating Instructions (Principal Investigator Only)
The Aneo System™ GUI is intended for use only by the Principal Investigator and not by the research subject. The Aneo System™ GUI is pre-installed on a computer at the clinical trial site. An internet connection is required for the computer in order for recorded data to be backed up. Note: the Aneo System™ GUI used in the clinical trial has been modified to prevent users from seeing what treatment zones are active.

11.1 Opening the GUI
1. Start the Aneo System™ GUI. Double-click the Aneo Device Management icon located on the desktop. The Aneo System™ GUI login screen will load.
2. Login to the Aneo System™ GUI. Enter the user name and password provided by SmileSonic and click the Login button.

11.2 Assigning the First Treatment
1. Connect device to computer. Use the USB cable to connect the Aneo System™ to a free USB port on the computer. Click the Refresh button in the Aneo System™ GUI. A device will appear in the Devices pane of the Aneo System™ GUI. Click on the device that just appeared. This will bring you to the review treatment screen.
2. Review previous treatment. Since this is a new device, there have been no previous treatments. By default, a previous treatment with a length of two days and zero complaints will be displayed. Ignore this previous treatment and click the Next button to continue to the setup screen.
3. Setup treatment. In the setup screen, select the next appointment date for the patient and continue to the confirmation stage by clicking the Update device button. Note: if the patient does not return for their next appointment, treatments on the device will be disabled one week after the next appointment date.
4. Confirm treatment. Confirm the treatment by entering your password and clicking the Confirm button. If the update is successful, a message will be displayed informing you that the device is programmed and can be unplugged. The device is now ready for the patient. If the update was not successful, try updating the device again.

11.3 Reviewing Previous Treatments and Assigning Subsequent Treatments
1. Connect device to computer. Use the USB cable to connect the Aneo System™ to a free USB port on the computer. Click the Refresh button in the Aneo System™ GUI. A device will appear in the Devices pane on the left of the Aneo System™ GUI. Click on the device that just appeared. This will bring you to the review treatment screen.
2. Review previous treatment. All previous treatments will be displayed at the bottom of the screen. Right-click on the most recent treatment and click "Save as PDF" to generate a report for that treatment. If a new treatment needs to be assigned, click the Next button to continue to the setup screen. Otherwise, you can unplug the device.
3. Setup treatment. In the setup screen, select the next appointment date for the patient and continue to the confirmation stage by clicking the Update device button. Note: if the patient does not return for their next appointment, treatments on the device will be disabled one week after the next appointment date.
4. Confirm treatment. Confirm the treatment by entering your password and clicking the Confirm button. If the update is successful, a message will be displayed informing you that the device is programmed and can be unplugged. The device is now ready for the patient. If the update was not successful, try updating the device again.

12. Treatment Schedule
Treatment consists of using the Aneo System™ for 20 minutes/day for the duration of the clinical trial. While the treatment is active, the Aneo System™ displays a countdown of the remaining treatment time. After 20 minutes, the Aneo System™ automatically stops the treatment and alerts the research subject that the treatment is complete.
13. Troubleshooting

13.1 Handheld Electronics

Handheld Electronics Damaged
If there is damage to the handheld electronics (e.g., LCD, buttons, etc.), do not use device and please return the device to the Principal Investigator for replacement.

Nothing Displayed on LCD
If nothing is displayed on the LCD while the device is on, reset the device by turning the device off and then back on again. If the problem persists, charge the battery. If the problem still persists, please return the device to the Principal Investigator for replacement.

LCD Freezes
If the LCD freezes and becomes unresponsive, reset the device by turning the device off and then back on again. If the problem persists, please return the device to the Principal Investigator for replacement.

13.2 Mouthpiece

Mouthpiece Damaged
If there is damage to the mouthpiece, do not use device and please return the device to the Principal Investigator for replacement.

13.3 Treatment Cable

Treatment Cable Damaged
If there is damage to the treatment cable, do not use device and please return the device to the Principal Investigator for replacement.

13.4 Coupling Gel

Adverse Reaction
If any adverse reaction to the coupling gel occurs, please contact the Principal Investigator.

Insufficient Coupling Gel
If there is insufficient coupling gel, please contact the Principal Investigator to obtain more coupling gel.

13.5 Charger

Battery Does Not Recharge
If the battery does not recharge, please return the device to the Principal Investigator for replacement.

Charger Damaged
If there is damage to the charger, do not charge the device’s battery and please return the charger to the Principal Investigator for replacement.

13.6 Any Other Failure in Operation

If the device fails to operate as intended, reset the device by turning the device off and then back on again. If the problem persists, please return the device to the Principal Investigator for replacement.

14. Care and Handling

The Aseo System™ contains complex electronic and ultrasonic technology and should be handled with care. In order to avoid possible damage, it is important to note the following:

- The Aseo System™ should be operated at room temperature conditions expected in a typical home environment (10°C to 40°C). If the Aseo System™ is stored or transported in temperatures outside this range, allow the device to return to room temperature before operating.
- Special care should be taken when handling the mouthpiece. Avoid bending or stretching the mouthpiece more than is required to place it in your mouth. Excessive force can cause internal damage to the mouthpiece.
- Before each treatment, inspect each of the Aseo System™ components for cracks or signs of damage. If there are any cracks or damage, do not use the device and please return it to the Principal Investigator.
- Protect the Aseo System™ from impact and exposure to moisture, liquid spills, sand, dirt, or debris.
- Protect the Aseo System™ from extreme hot or cold temperatures.
- Do not use cleaning agents or solvents (including mouthwash or alcohol) on any of the components of the Aseo System™.

- Never immerse the Aseo System™ handheld electronics in water.
- To clean the handheld electronics, use a clean soft cloth, tissue, paper towel, or cotton swab.
- Remove any excess coupling gel from the mouthpiece using a clean damp, soft cloth or paper towel. Clean the mouthpiece with a manual toothbrush and toothpaste, using light pressure. Rinse the mouthpiece with cold tap water.
- Do not use a heat source to dry the mouthpiece. Let the mouthpiece dry at room temperature.
- Store the Aseo System™ in a clean and dry environment.
- Do not attempt to modify or repair the Aseo System™. There are no user serviceable parts inside the device.

15. Contact Information

The research subject should notify the Principal Investigator if:

- He or she has any adverse reaction, complication, or problem during treatment;
- The device malfunctions or develops a problem; or
- He or she does not have sufficient coupling gel to complete his or her therapy.

16. Device Manufacturer

SmileSonics Inc.
Suite 4-034 NINT Innovation Centre
11421 Saskatchewan Drive
Edmonton, Alberta, Canada T6G 2M9
info@smilesonics.com
780-641-1904

17. References


A6. Research Contribution Agreement Between The University of Toronto and Smile Sonica Inc, the Manufacturer of the Device

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SMILESONICA RESEARCH CONTRACT

This agreement (the "Agreement") is made in two original counterparts effective March 22nd, 2013 (the "Effective Date").

BETWEEN:

THE GOVERNING COUNCIL OF THE UNIVERSITY OF TORONTO
(the "University")

and

SMILESONICA INC.
Suite 4-034, NINT Innovation Centre
11421 Saskatchewan Drive
Edmonton, Alberta
T6G 2M9
("SmileSonica")

WHEREAS the University is conducting a research project entitled "The effect of low-intensity pulsed ultrasound (LIPUS) on orthodontic tooth movement" (the "Project") under the supervision and direction of Dr. Kevin Knowlton of the University's Faculty of Dentistry (the "Principal Investigator") and of Dr. Bryan Tompson of the University's Faculty of Dentistry (the "Co-Investigator") together referred as the "Investigators";

AND WHEREAS the University requires low intensity pulsed ultrasound devices for use on the Project;

AND WHEREAS SmileSonica exclusively owns the technology and is the manufacturer of a device called the Aevos System™ for emitting low intensity pulsed ultrasound, and is willing to support the Project by contributing the Aevos System™ devices (the "Devices"), the Aevos Device Management software (the "Software") installed on a laptop computer (the "Laptop") needed for the conduct of the Project;

AND WHEREAS SmileSonica and the University hereby agree as follows:

1.0 THE PROJECT

1.1 Project. Subject to, and in accordance with, the terms and conditions in this Agreement, the University will perform the Project as described in and in accordance with the provisions of protocol number PR-0031 "The Effect of Ultrasound on Orthodontic Tooth Movement" ("Protocol") set out in the attached Appendix "A" under the supervision and direction of the Investigators, together with such personnel as the University may assign. The University does not guarantee nor is it obliged to deliver specific results. The University makes no warranties or representations regarding its ability to achieve, nor shall it be bound to accomplish, any particular research objective or results.
1.2 **Contribution.** SmileSonica will provide at no cost to the University, the Devices, the Software and the Laptop needed for performance of the Project, including shipping and handling, as further described in Appendix “B”. The Devices, the Software and the Laptop remain the property of SmileSonica at all time. The Devices must be returned to SmileSonica at the end of the treatment of each patient, or at the request of SmileSonica. The Software and the Laptop must be returned to SmileSonica at the end of the Project, or at the request of SmileSonica. The Devices, the Software and the Laptop are not to be altered or disassembled in any way during the Project while in the possession of the University.

1.3 **Confidential Information.** Except as otherwise permitted pursuant to this Agreement, no party to this Agreement shall disclose to any third party either the terms of this Agreement or any information disclosed to it under this Agreement. If either party discloses confidential information to the other party under this Agreement, the disclosing party will identify such information as “confidential” in writing at the time of its transmittal, or so reduce to writing within ten (10) days thereafter (“Confidential Information”). The receiving party will safeguard and not disclose such Confidential Information to third parties for a period of five (5) years of receipt from the disclosing party. Confidential Information will not include information that:

(a) the receiving party can demonstrate through documentary records to have been known to the receiving party prior to its receipt from the disclosing party;

(b) is or becomes publicly available other than through an act or omission of the receiving party or any of its employees;

(c) the receiving party lawfully obtained from sources under no obligation of confidentiality;

(d) is required to be disclosed under the requirement of judicial process, regulatory authority or law, and the receiving party has given thirty (30) days advance notice to the disclosing party.

Notwithstanding anything contained herein, each party may disclose Confidential Information to its officers, employees, consultants, agents, and students on a need-to-know basis in relation to the Project, provided that such persons agree to be bound by the terms at least as restrictive as those contained herein.

**SmileSonica Data** (as defined in section 2.1) generated by this Project shall be considered confidential of SmileSonica, except to the extent that it is included in a publication, pursuant to section 2.3 of this Agreement.

The University and the Investigators shall return to SmileSonica, upon request, the materials, samples, graphics, writings, and information in other tangible forms, containing any confidential information provided by SmileSonica, and any copies of such confidential information, excepting one archival copy to be retained by the University for purposes of observing compliance with this Agreement.

No license, express or implied, to use confidential information is granted under this Agreement other than to use confidential information in the manner and to the extent authorized by this Agreement.

### 2.0 RESEARCH RESULTS

#### 2.1 Definitions:

“Confidential Information” is information as specified as confidential in section 1.3 of this
Agreement.

"Intellectual Property" means inventions, ideas, discoveries, innovations, devices, data, mechanisms, substances, technologies, works, trade secrets, know-how, formulae and methods, including improvements, whether or not protectable by patent, copyright or other intellectual property rights.

"Invention" means any Intellectual Property conceived, reduced to practice, made or developed, in whole or in part, by the University, Investigators or those for whom they are responsible at law, in the conduct of the Project that relates in any way to the Project drug/device, including its administration or use, alone or in combination with any other drug or device, and any related assay.

"Inventor" means a person who creates, in whole or in part, an invention.

"Tangible Property" means biological samples, genomic data, read-outs from equipment, photographs and other data not specified as University Data or SmileSonica Data.

"University Data" means source documents, original raw data, x-rays, models and subject medical records.

"SmileSonica Data" means completed Project case report forms and reports, copies of patient reports and raw data, copies or duplicates of materials including x-ray, casts and models from the Project, and the gender and birth year of the patient. The SmileSonica Data must be linked to a unique patient identifier (a unique number or initials), and where private patient information is removed or redacted as required.

2.2 Ownership:

a) Intellectual Property that any party owned prior to execution of this Agreement, or develops independently of the Project and any other party’s Confidential Information, is that party’s separate property and is not affected by this Agreement. No party has any claims to or rights in such Intellectual Property of the other parties.

b) Within thirty (30) days of becoming aware of any Invention, the University and the Investigators will disclose the Invention to SmileSonica, on a confidential basis, with a full written description. University and the Investigators will make available to SmileSonica, at SmileSonica request and expense, all work, reports, writings, ideas, designs, methods, computer software, and data, recorded in any form, that relates to the Invention.

c) SmileSonica will own Inventions created in the University and the Investigators conduct of Project which are necessarily made with or directly derived from Project drug/device, or concerning methods of using Project drug/device. Subject to section 2.2, the sole or joint ownership of all other Inventions will be determined in accordance with applicable patent law, regardless of whether the Invention is patentable. SmileSonica and the University may independently exploit joint Inventions without compensation, liability or other obligation to the other party, subject, however, to SmileSonica’s option to license the University’ rights in section 2.2 d) below of this Agreement.

d) For University-owned Inventions or joint Inventions, University will grant SmileSonica
an exclusive option, without fee, exercisable within sixty (60) calendar days following written notice of an Invention, to obtain an exclusive or nonexclusive, worldwide, royalty-bearing commercialization license, upon reasonable commercial terms and conditions, to all rights, title and interest that University may have or obtain in that Invention. This license will include the right to sublicense, make, have made, use, and sell the Invention or products incorporating the Invention. Upon SmileSonica's exercise of its option with regard to any particular invention, University and SmileSonica will negotiate in good faith for up to ninety (90) days in an attempt to reach a license agreement satisfactory to both parties. If an agreement is not reached by the end of that period, SmileSonica's rights to that Invention will expire, and University may license Invention to third-parties without obligation to SmileSonica. Any such license agreement will include reimbursement to University for its reasonable past and SmileSonica-approved future costs of filing, prosecuting, and maintaining any patent or other intellectual property applications related to licensed Invention. If SmileSonica does not exercise its option on University' ownership of joint Inventions, University may exercise an option to license SmileSonica's ownership share of that Invention, under the symmetrical terms of this section.

e) At SmileSonica's request and expense, where applicable, the University and Investigators will execute, or cause to be executed by its Inventors, all documents and perform all acts deemed necessary by SmileSonica to evidence SmileSonica's ownership of Inventions, obtain patents in any country, and otherwise protect SmileSonica's interests in Inventions. SmileSonica has the exclusive right to incorporate any Project data in any regulatory filing concerning any Project drug/device. For joint Inventions, SmileSonica may file jointly to file joint patent or other intellectual property applications at its own expense within ninety (90) calendar days following written notification of that invention and will provide the University with copies of any applications it files and of any written communications to or from the applicable patent or other intellectual property office regarding any such Invention. If SmileSonica does not file such application(s) within ninety (90) calendar days after disclosure of the Invention to SmileSonica by the University, the University will obtain the rights to joint filing for such Invention.

f) The University shall own all the University Data. SmileSonica owns all SmileSonica Data. SmileSonica and the University jointly own Tangible Property. The parties may freely use data they own subject to patient consent and Research Ethics Board approval. SmileSonica has no right to the University' pre-existing biological samples, genomic database, or other proprietary database except for purposes that relate directly to the Project.

2.3 Dissemination.

The University and Investigators may, after the earlier of twelve (12) months following completion of the Project or SmileSonica obtaining marketing clearance from Health Canada, freely publish and disseminate the results of his/her investigative findings hereunder and shall solely determine the authorship and contents (including scientific conclusions and professional judgments) of any such publication or dissemination ("Publication"), subject always to the following terms and conditions:

a) If the University and Investigators wish to make a Publication the University and Investigators shall provide to SmileSonica the manuscript to be submitted for
Publication (the “Manuscript”) at least thirty (30) days prior to the planned date of submission for Publication.

b) Within thirty (30) days of receiving the Manuscript, SmileSonica shall advise the University and Investigators in writing:

i. that the Manuscript contains SmileSonica’s confidential information; or

ii. that the Manuscript contains information for which SmileSonica wishes to obtain patent protection or any other intellectual property protection.

c) If SmileSonica responds pursuant to section 2.3 b) i., the University and Investigators shall remove from the Manuscript, prior to Publication, all information reasonably identified by SmileSonica as confidential. If SmileSonica does not identify and require the removal of any confidential information pursuant to section 2.3 b) i, the same may be included in the Publication.

d) If SmileSonica responds pursuant to section 2.3 b) ii., the University and Investigators shall, delay Publication of the Manuscript for an additional ninety (90) days from when SmileSonica responds pursuant to section 2.3 b) ii.

e) SmileSonica may comment upon, but may not make or require any changes to the Manuscript, except as noted in sections 2.3 c) and 2.3 d) above.

f) If SmileSonica fails to advise the University and Investigators within thirty (30) days of receipt of the Manuscript, the University and Investigators may proceed with the Publication of the Manuscript.

g) SmileSonica personnel shall be acknowledged in accordance with customary scientific practice.

h) Notwithstanding section 2.2 e), the University and Investigators shall, subject to any requirement of any publisher of a Publication to assign copyright to such publisher, own the copyright on Publications. the University and Investigators grants SmileSonica a perpetual, irrevocable, royalty free license to make and distribute copies of any Publications disclosed to SmileSonica pursuant to section 2.3 a), to the extent University and Investigators has such rights, and any other copyrightable material referred to in this Section 2.3 h) which constituted a deliverable of the University and Investigators to SmileSonica pursuant to the Protocol.

i) Notwithstanding sections 2.3 a) to 2.3 h), SmileSonica retains all rights to freely publish and disseminate the results of the investigative findings hereunder and shall solely determine the authorship and contents (including scientific conclusions and professional judgments) of any such publication or dissemination. University and Investigators retain the right to make the first academic publication in a peer-reviewed scientific journal for a period of 1 year.

2.4 Results. The University will provide SmileSonica with a copy of the “SmileSonica Data” and the results of the Project within a reasonable time, but so later than two (2) months, following
the completion of the Project. All such information provided to SmileSonica shall be considered Confidential Information.

2.5 Similar Research. Except for section 1.3 nothing in this Agreement will be construed to limit the freedom of the University or of its researchers from engaging in similar research under agreements with parties other than SmileSonica.

3.0 TERM AND TERMINATION

3.1 Term. This Agreement will enter into force as of the Effective Date and will terminate on the earliest of Project completion or two years from the Effective Date, unless sooner terminated in accordance with section 3.2 below, or upon the written agreement of the parties.

3.2 Termination. Either party may terminate this Agreement upon ninety (90) days written notice to the other party.

3.3 Effect of Termination. The provisions of sections 1.3, 2.1, 2.2, 2.3, 4.1, 4.2, 4.3 and 4.4 will survive termination or expiration of this Agreement in accordance with their terms.

4.0 MISCELLANEOUS

4.1 Disclaimer.

The University and Investigators shall carry out the Project in accordance with this Agreement, the Protocol and all non-conflicting written instructions provided by SmileSonica, but do not promise success in achieving any desired result. In addition, The University and Investigators make no representations or warranties regarding any merchantability of the Project results, or the fitness of the Project results for any particular purpose. The University and Investigators shall not be liable for any direct, consequential or other damage suffered by SmileSonica or others resulting from the development or use of the Project results. The University and Investigators do not warrant that the Project results or any part thereof or any aspect of the same shall be capable of receiving statutory protection.

Except in the case as per section 1.2 and as outlined in section 4.2 the University and Investigators shall have no liability whatsoever to SmileSonica in connection with the Devices, including damage to the Devices however caused, or any Project results described in any report or other information that may be provided to SmileSonica.

4.2 Limitation of Liability.

SmileSonica will indemnify and save harmless the University and its employees, students and agents against all costs, suits or claims resulting from: (i) the University's use of the Devices unless caused by the willful misconduct or negligent act or omission of personnel of the University, (ii) any third party claims of infringement of any copyright or patent related to the University's use of the Devices, or, (iii) the use by SmileSonica or its affiliates, its customers or licensees of any information or intellectual property developed under this Agreement. The University will indemnify and save harmless the SmileSonica against all costs, suits or claims on account of injuries (including death) to persons participating in the Project or to damage to
University property caused by the willful or negligent act or omission of personnel of University during the performance of this Agreement.

4.3 Insurance

SmileSonic's carries general liability insurance and product liability insurance, each with limits of at least $1,000,000 per occurrence and $1,000,000 in the aggregate. SmileSonic's insurance includes blanket contractual liability, covers the Project and is not materially encumbered by existing claims. The minimum amounts of insurance coverage required shall not be construed to create a limit of SmileSonic's liability with respect to its indemnification. SmileSonic shall maintain such coverage for the duration of this Agreement and if the policy is claims-made, for two (2) years thereafter. SmileSonic shall provide certificates of insurance to the University upon request. SmileSonic shall notify the University within thirty (30) days of any notice of cancellation or non-renewal of, or material change in, or claim against, its insurance coverage.

The University carries general liability insurance and product liability insurance, each with limits of at least $1,000,000 per occurrence and $1,000,000 in the aggregate. The University's insurance includes blanket contractual liability, covers the Project and is not materially encumbered by existing claims. The minimum amounts of insurance coverage required shall not be construed to create a limit of the University's liability with respect to its indemnification. The University shall maintain such coverage for the duration of this Agreement and if the policy is claims-made, for two (2) years thereafter. The University shall provide certificates of insurance to SmileSonic upon request. The University shall notify SmileSonic within thirty (30) days of any notice of cancellation or non-renewal of, or material change in, or claim against, its insurance coverage.

4.4 Use of Names.

The use by any party of the name, trademark, trade name, logo, or any adaptation thereof, of any other party in any publication, press release, advertisement, announcement, promotional material, or promotional activity relating to the Project requires the prior written consent of the affected party, subject, however, to the following:

a) SmileSonic may, without prior consent, identify the University as an entity that participated in the Project, and identify the Investigators as conducting the Project at the University. This paragraph does not apply to information of sub-investigators or other Project personnel;

b) The University and Investigators may, without prior consent, disclose their participation in the Project (restricted to the name of SmileSonic, name of the Project, and protocol number) in any University annual report, or as required by law, Court order, or regulation;

4.5 Notices. Notices under this Agreement will be sent to the parties as follows or to such other person as a party may designate in writing:

(a) to the University:

i. to the Investigators (for technical and scientific matters):

Dr. Kevin Knowlton - Principal Investigator
Faculty of Dentistry
University of Toronto
124 Edward Street
Toronto, ON
M5G 1G6- Canada
Kevin.knowlton@mail.utoronto.ca | 416-979-4900

Dr. Bryan Tompson - Co-Investigator
Faculty of Dentistry
University of Toronto
124 Edward Street
Toronto, ON
M5G 1G6- Canada
Bryan.tompson3@dentistry.utoronto.ca | 416-979-4900 ext. 4605

ii. for legal and administrative matters:

Lauren Gogo
Contracts Officer
Innovations & Partnerships Office, University of Toronto
Banting Institute
100 College Street, Suite 413,
Toronto, Ontario
M5G 1L5 – Canada
Lauren.gogo@utoronto.ca | 416-978-1512

(b) to SmileSonica:

Cristian Scurtescu
President and CEO
Suite 4034, NINT Innovation Centre
Edmonton, Alberta, Canada, T6G2M9
cristian.scurtescu@smilesonica.com
Phone 780 641 1964

4.6 **Independent Parties.** The parties are independent parties and nothing in this Agreement will constitute either party as the employer, principal or partner of or joint venturer with the other party. Neither party has any authority to assume or create any obligation or liability, either express or implied, on behalf of the other party.

4.7 **No Assignment.** Except as provided for in section 2.2, neither party may sell, assign, encumber, licence or otherwise transfer any of its rights, duties or obligations under this Agreement without the prior written consent of the other party, which consent may not be unreasonably withheld.

4.8 **Successors.** This Agreement binds and survives to the benefit of the parties hereto and their respective heirs, successors and permitted assigns.

4.9 **Interpretation.** This Agreement will be governed by and construed in accordance with the laws of the Province of Ontario in Canada. In the event that a court of competent jurisdiction holds any provision of this Agreement to be invalid, such holding will have no effect on the remaining
provisions of this Agreement, which will continue in full force and effect. Headings are used for convenience only and will not be used to interpret the provisions of this Agreement.

4.10 **Time of Essence.** Time shall be of the essence hereof.

4.11 **Entire Agreement.** This Agreement is the entire agreement of the parties with respect to its subject matter and no change or modification will be valid unless it is in writing and signed by both parties.

**IN WITNESS WHEREOF** by signature of their respective authorized officers, the parties agree to be bound by the terms of this Agreement.

**THE GOVERNING COUNCIL OF THE UNIVERSITY OF TORONTO**

Name: Lino DaFacendis  
Title: Director, Partnerships

![Signature]

**April 18, 2012**

**SMILESONICA INC.**

Name: Cristian Scurtescu  
Title: President and CEO

![Signature]

**April 2nd, 2013**

**Acknowledgement:**

I, the **Principal Investigator**, having read this Agreement, hereby agree to act in accordance with all the terms and conditions herein and applicable **University** policies, and further agree to ensure that all **University** participants are informed of their obligations under such terms and conditions.

**Name:** Kevin Knowlton

**March 28, 2013**

I, the **Co-Investigator**, having read this Agreement, hereby agree to act in accordance with all the terms and conditions herein and applicable **University** policies, and further agree to ensure that all **University** participants are informed of their obligations under such terms and conditions.

**Name:** Bryan Thompson

**March 28, 2013**
Appendix B

B1. Sample Size Calculation

Comparing the means of two normally distributed samples of equal size with a significance of 0.05 and power of 80%.  (From: Rosner, B. Fundamentals of Biostatistics. 7th ed. 2011.)

\[ n = \frac{2\sigma(1.96 + 0.84)^2}{(\mu_2 - \mu_1)^2} \]

where:

\( \mu_1 = \) the average rate of tooth movement in humans which is approximately 1.11mm/month with a standard deviation of \( \sigma = 0.43\text{mm/month} \) (Table 1)

\( \mu_2 = \) an estimated 50% increase in tooth movement with ultrasound based off of studies in dogs, which would be 1.66mm/month

so:

\[ n = \frac{2 \times 0.43(1.96 + 0.84)^2}{(1.66 - 1.11)^2} = 9.58 = 10 \text{ subjects in each group} \]

Table 1. Average orthodontic tooth movement in humans.