Title
Clinical and histological comparison of incisions in animal tissue made with scalpel, carbon dioxide laser, diode laser, Nd:YAG and Er,Cr:YSGG lasers

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Clinical and histological comparison of incisions in animal tissue made with scalpel, carbon dioxide laser, diode laser, Nd:YAG and Er,Cr:YSGG lasers

by

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THESIS

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MASTER OF SCIENCE

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Clinical And Histological Comparison of Incisions In Animal Tissue Made With Scalpel, Carbon Dioxide Laser, Diode Laser, Nd:YAG And Er,Cr:YSGG Lasers

By

Ewa Konopka, DDS

Abstract

Objective: To test the null hypothesis that the incision properties of four different lasers: carbon dioxide, diode, Nd:YAG, and Er,Cr:YSGG are comparable clinically and histologically to each other, and the stainless steel scalpel blade incision.

Materials and Method: 30 incisions were made in fresh pig mandibles, in the keratinized oral mucosa, with the stainless steel scalpel blade, and the four lasers tested, 6 incisions for each treatment. The four lasers parameters were set according to each laser manufacturers' instructions for the soft tissue incision. Histological examination (H&E stain) assessed depth of incision, width of incision, lateral coagulation, depth of coagulation, and time to complete each incision. One-way ANOVA approach with a Bonferroni or Scheffe posttest analysis for the P value, with the P value set at <0.05 was used to analyze data.

Results: The scalpel incision induced no lateral or deep coagulation. The Nd:YAG laser exhibited the most lateral and deep coagulation, as well as the most width of incision. The Er,Cr:YSGG laser and the carbon dioxide lasers differed statistically in their depth of incision, having lesser depth, from the diode and the Nd:YAG lasers.

The incision time was the least with the scalpel, and the Nd:YAG laser took the most time to incise the soft tissue.

Conclusion: The findings support choosing lasers over the scalpel for the soft tissue incision if hemostasis and coagulation is clinically desired in the chosen procedure. The use of the stainless steel scalpel blade incision is more favorable than any type of laser if primary closure of the soft tissue is needed. Lasers time for incision in the soft tissue takes longer than the scalpel due to laser-tissue interaction effects of lasers.

Keywords: oral soft tissue incision with lasers, stainless steel scalpel blade, carbon dioxide laser, diode laser, Nd:YAG laser, Er,Cr:YSGG laser, ablation, necrosis, thermal damage, coagulation zones.

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INTRODUCTION

There is a paucity of current studies evaluating the soft tissue effects of lasers available for use in dentistry. The gold standard of soft tissue incision is the stainless steel scalpel blade. A scalpel is easy to use, accurate, with minimal damage to the tissues, but its downside is a lack of hemostasis in highly vascular oral soft tissues (10). Stainless steel scalpel blade incisions in soft tissues do not present with loss of surface tissue, and there are no thermal and coagulation effects (1, 7). Familiarity with the scalpel blade contributes to its ease of use, and the clinician's comfort in predicting tissue-scalpel interaction.

Lasers are well known for their coagulation ability, with some studies claiming better wound healing properties, decreased inflammatory response, and lesser wound contraction and scarring (10, 11, 12). One of the results of laser energy-tissue interaction is the possible thermal damage (4) that may lead to soft tissue necrosis and coagulation adjacent to laser incisions (8, 5).

During the late 1990s and the early 2000s, several histological studies were conducted comparing scalpel incisions with the carbon dioxide laser set at different power settings, in a continuous mode (4, 6, 7, 8, 9, 11). Some of these studies used various animal models, whereas others utilized human biopsy samples.

Wilder-Smith (4) investigated histologically the zones of soft tissue damage comparing a scalpel and three different power settings of the carbon dioxide laser (1, 4, and 12 Watts) in fresh pig mandibles.

Our study utilizes the Wilder-Smith animal tissue model, expanding it to include the four types of lasers presently used in dentistry: carbon dioxide laser, diode laser, Nd:YAG, and Er,Cr:YSGG lasers. There are no current studies comparing scalpel incisions to the varied types of lasers. The lasers settings in our study followed the manufacturers' instructions, as they would be used in the real life clinical situations to incise oral soft tissue.

Hypothesis

The aim of this study is to test the null hypothesis that the incision properties of four different lasers: carbon dioxide, diode, Nd:YAG, and Er,Cr:YSGG are comparable clinically and histologically to each other, and the stainless steel scalpel blade incision.

The histological parameters tested were:

- The width of incision
- The depth of incision
- Lateral coagulation
- The depth of coagulation

The clinical parameters tested were:

- Time of incision
- Clinical appearance of incision

Lasers

LASER is an acronym for Light Amplification by Stimulated Emission of Radiation. Albert Einstein first described stimulated emission at the beginning of the twentieth century, and this concept was brought to use in the 1960s with Maiman's invention of a ruby laser (1, 2).

All laser types have four main elements: an active medium, a pumping unit, optical feedback elements, and the auxiliary units, such as the cooling system (3). The distinguishing feature of each laser type is its wavelength, which depends on the properties of the active medium (1, 2, 3). The active medium can be a solid, liquid, gas, or semiconductor. The laser types commonly used in dentistry, and their corresponding active medium are as follows: carbon dioxide laser (gas); diode laser (semiconductor); Nd:YAG laser (solid); Er,Cr:YSGG laser (solid). A laser medium needs to have a potential for large number of atoms in the higher energy state than in the ground state, so the process of stimulated emission creates two photons with the same wavelength, as the higher energy photon is stimulated by another photon of precisely the same wavelength (1, 2).

In the process of irradiating the tissue with laser light, the laser beam diminishes as it moves into soft tissue, loosing its energy through absorption. The strength of absorption depends mainly on laser wavelength, and specific molecules (water or non-water) that comprise the tissue.

When the laser wavelength is highly absorbed in water (such as carbon dioxide and Er,Cr:YSGG lasers), it does not penetrate deeply into the soft tissues, as the surface cells have high water content. The laser wavelengths with no water affinity, but highly absorbed by hemoglobin and melanin, penetrate deeply into the soft tissues (diode and Nd:YAG lasers) (1, 2).

Another important feature of any laser type is its mode of operation: continuous wave or pulsed (2). Gas active medium lasers, such as carbon dioxide, work in a continuous wave mode, and the light beam is emitted with constant power. Solid state lasers, such as Nd:YAG and Er,Cr:YSGG, give pulsed oscillation mode. The radiation comes out as periodic light pulses, 1 to 10 seconds long, at a frequency from 10 Hz to 10 kHz. The pulsed mode offers a large power output within short time intervals. Most pulsing is designed into the laser (2). The mode of operation controls the power density of a given type of a laser. Laser's ability to incise, vaporize, and coagulate various tissues of the body is determined by power density (Watts/cm), and it indicates the rate of tissue removal in the spot (2). However, a laser is chosen for its specific peak absorption rates rather than for its power density. This brings us back to the importance of water and non-water absorption of the laser light in the oral soft tissue, as each laser wavelength has a different absorption of water and non-water molecules.

Types of Lasers Commonly Used in Dentistry

The carbon dioxide laser has a gas state active medium. It consists of a mixture of carbon dioxide, molecular nitrogen, and an additive, such as helium. Carbon dioxide laser operates at 10.6 microns (10,600 nm) wavelength, which is in an infrared region, and it is strongly absorbed by water. Carbon dioxide laser functions mainly in a non-contact mode, and in a continuous wave mode (1, 2).

The diode laser has a semiconductor active medium, which is different than a crystal in a solid-state laser, because it has no individual atoms with energy levels to be stimulated. Aluminum gallium arsenide (AlGaAs) lasers operate in the near-infrared spectrum, around the 800 to 900 nm wavelength. They function in a continuous wave mode. Diode laser penetrates deeply into the soft tissue, due to hemoglobin absorbing laser energy (1).

The Nd:YAG laser (neodymium:yttrium aluminum garnet) is a solid state laser. The active medium is yttrium aluminum garnet crystal in which some of yttrium ions are replaced by neodymium ions (Nd doped YAG). It has a 1.06 microns (1064 nm) wavelength. The Nd: YAG laser can operate in either the continuous wave or pulsed modes, but usually it is pulsed down in microseconds. The Nd:YAG laser penetrates deep into the soft tissues because of the high absorption of its wavelength by hemoglobin, and minimal absorption in water (1, 3).

The Er,Cr:YSGG laser (erbium, chromium: yttrium scandium gallium garnet) has a solid-state active medium. Its yttrium scandium gallium garnet crystal, sensitized with chromium is doped with erbium ions. It operates at a 2790 nm wavelength, in a pulsed mode. The Er,Cr:YSGG laser is air-, and water-cooled, and its light is strongly absorbed by water in the soft tissue (3).

Laser-Tissue Interaction

The monochromatic quality of the laser light allows it to be utilized to surgically incise tissue. However, the process of laser radiation incising soft tissue alters the incision site based on the absorption spectrum of the particular molecules, such as water, hemoglobin, melanin, and collagen. The laser energy also releases heat, which denatures proteins, coagulates, and vaporizes the tissue. Laser incisions, regardless of the wavelength, present with a pattern of zones of effect in the soft tissue, caused by absorption of the electromagnetic energy (2, 19). The zones of laser-tissue interaction are important in the wound healing and inflammatory processes, and they were shown by Pogrel et al. (7, 8) to heal by secondary intention due to tissue necrosis.

Zones of laser-tissue interaction

1) Necrosis/ablation zone
2) Coagulation zone
3) Stasis zone
4) Edema zone

The ablation zone with its tissue necrosis creates loss of soft tissue in the incision site, and the damage is irreversible. The coagulation zone with its protein denaturation creates a hemostatic effect on the highly vascular oral soft tissue, and the changes are irreversible. The coagulation zone extends around the ablation defect in the lateral and apical direction. The stasis and edema zones immediately follow the coagulation zone, and the tissue damage is reversible in the healing process. During the histologic examination, the coagulation zone includes the stasis and edema zones (19). In1991, Dederich (18) described three histologic zones of thermal effect resulting from the carbon dioxide laser use in the soft tissue: 1) an outer zone of the ablation cavity caused by the vaporization of the tissue; 2) a middle zone of tissue coagulation; 3) an inner zone of thermal damage (stasis and edema zones) with the potential for repair.

The width and the depth of the incision, with its coagulation, stasis and edema zones, performed with different types of lasers will vary based on the wavelength and subsequent water absorption of each laser. Therefore, due to their water absorption in the soft tissue, the carbon dioxide and Er,Cr:YSGG lasers have the shallowest incision depth and the smallest tissue damage zones. The diode and Nd:YAG lasers present with the deepest incision depth, and greater tissue damage zones, as their wavelength is strongly absorbed by hemoglobin.

Wilder-Smith et al. (4) used the carbon dioxide laser in a continuous mode to make incisions in fresh pig mandibles. They found the incision depth of the carbon dioxide laser to depend on the power setting (1W, 4W, and 12W) and ranged from 164 to 410 microns.

The incision width for the carbon dioxide laser ranged from 300 to 722 microns. The tissue coagulation zone extended approximately 60 microns into the soft tissues (4). Vaderhobli et al. (5) measured the width and depth of tissue removed with a microsecond-pulsed carbon dioxide laser, as well as lateral and deep coagulation of the soft tissue incision in fresh pig mandibles. The study compared these parameters with the historical data. It concluded that the width of incision, the lateral coagulation, and the deep coagulation were the same. However, the depth of incision was significantly deeper than the historical data for the carbon dioxide laser.

Perry et al. (20) evaluated different probe diameters of the Nd:YAG laser used to excise bovine soft tissue in vitro. They found the depth of incision ranged from 0.19 mm to 0.49 mm; width of incision ranged from 0.63 mm to 0.79 mm. Lateral and deep coagulation ranged from 0.27 mm to 0.62 mm. The Pogrel study (7) in rats reported the mean width of tissue necrosis zone for the 20W carbon dioxide laser to be 190 microns, with a range of 110 to 300 microns. The stainless steel scalpel blade incision exhibited no tissue coagulation zone, and the histologic presentation showed no loss of surface epithelium, and a blood clot in the surgical defect.

In a histologic evaluation of human excisional biopsy samples taken with the carbon dioxide laser, Pogrel et al. (8) measured soft tissue necrosis adjacent to the laser incision. The tissue necrosis was up to 100 microns lateral to the actual carbon dioxide laser wound. The tissue coagulation zone was visible next to the zone of tissue necrosis, and it extended up to 500 microns.

Pogrel et al. (8) concluded that carbon dioxide laser causes similar or greater amount of tissue damage than the stainless steel scalpel blade.

Kirschbaum et al. (14) assessed the tissue penetration of Nd:YAG laser in lung resection in pigs. They found the mean necrosis depth to be 1.74 mm. Van Nimwegen et al. (15) experimented with the Nd:YAG laser in canine prostate using contact and non-contact modes. They found that the Nd:YAG laser contact mode incision caused necrosis of tissue which left a crater underneath the fiber tip. The crater depth created in a contact mode in the soft tissue ranged from 1.3 mm at 5 seconds to 3.5 mm at 20 seconds pulses. The tissue coagulation zone underneath the crater floor was 0.9 mm for the Nd:YAG laser in a contact mode, and 4.7 mm for the Nd:YAG in a non-contact mode (15).

Seoane et al. (16) researched the width of tissue damage with the Er,Cr:YSGG laser used in excisional biopsies of human oral leukoplakia. They found the coagulation zone lateral to the incision to be 26.6 microns. They concluded that the wide margins in biopsy using the Er,Cr:YSGG laser are not necessary, as opposed to the wider margins of healthy tissue required (1 to 3 mm) when using any other type of laser: diode, carbon dioxide, or Nd:YAG.

Giannelli et al. (17) experimented with comparative evaluation of photoablation on gingival biopsies taken from periodontally involved patients using diode, Er,Cr:YSGG, Nd:YAG, and the carbon dioxide lasers. The Er,Cr:YSGG laser showed incomplete ablation of gingival epithelium, and a lack of hemostasis.

The carbon dioxide laser exhibited complete removal of the gingival epithelium, with a diffuse, heat-induced coagulation of the connective tissue. The Nd:YAG and diode lasers showed similarly complete removal of the gingival epithelium with an effect of collapsing microvessels, which increased hemostasis (17).

Clinical Examples of Laser-Tissue Interaction in Dentistry

LANAP (Laser Assisted New Attachment Procedure) is a combined therapy that uses Nd:YAG laser for surgical pocket epithelium removal, followed by scaling and root debridement with piezo instruments. After that, Nd:YAG laser is used the second time to form a fibrin clot for tissue stabilization. The treated teeth undergo an occlusal adjustment and the splinting of teeth is performed as needed (21).

In 2007, Yukna et al. (21) showed, with histologic evaluation in humans, that LANAP can be associated with periodontal regeneration of the new cementum, new connective tissue, and new bone attaching to the previously diseased root surface. Six pairs of single-rooted teeth with an isolated moderate to severe periodontal defect, and planned for extraction due to the restorative plan, were studied. All teeth received scaling and root planing, with the control teeth treated with the Nd:YAG laser. After 3 months, test and control treated teeth were removed for histologic examination. The LANAP treated teeth had greater mean probing depth reduction (4.7 mm vs 3.7 mm) than the control.

All six LANAP treated teeth showed periodontal regeneration. Five of six control teeth showed long junctional epithelium, and no evidence of new attachment or regeneration (21). Nevins et al. (22, 23) analyzed histologically the healing following LANAP around ten human teeth after en bloc biopsy. The data showed five teeth exhibiting periodontal regeneration. Harris et al. (24) conducted a split-mouth, randomized clinical trial comparing LANAP, flap surgery using Modified Widman technique, scaling and root planing, and coronal debridement. The results at 6 and 12 months showed pocket depth reduction similar for LANAP and Modified Widman Flap. LANAP resulted in greater reduction of sites bleeding upon probing, as well as less post-operative discomfort for the patient.

The LANAP protocol utilizes Nd:YAG laser in a surgical treatment of the periodontal disease. However, other types of lasers (diode and Er,Cr:YSGG) are reported in the literature as to their effects on the treatment of periodontal disease, as an adjunct to non-surgical periodontal therapy. The systematic review and meta-analysis by Smiley et al. (25) concluded that the evidence is moderate to low in favor of scaling and root planing with an addition of any laser type: diode, Er,Cr:YSGG, or Nd:YAG. Gokhale et al. (26) evaluated the adjunctive use of a diode laser with mechanical debridement in the periodontal flap surgery. The clinical parameters in the test and control groups did not reach statistical significance, but patient discomfort was lesser as measured by the Visual Analog Scale.

In summary, there is no standard operating protocol for the use of a laser in the non-surgical and surgical periodontal therapy, which makes results difficult to compare across laser types. The Nd:YAG, Er,Cr:YSGG, and diode lasers can be used to treat moderate to severe periodontitis, patients with bleeding disorders, and patients phobic to surgery (13). McCawley et al. (32) investigated the effect of the Nd:YAG laser in LANAP protocol on chronic periodontitis microbiota. The results showed that LANAP treated patients were 85 percent free of periodontal pathogens, such as *Porphyromonas gingivalis, Tannerella forsythia, Fusobacterium* species, and others. 100 percent of patients treated only with ultrasonic debridement still had periodontal pathogens present in subgingival pockets, the same as at baseline.

The LAPIP (Laser Assisted Peri-Implantitis Procedure) is a LANAP protocol modified to treat ailing and failing implants with the Nd:YAG laser. Nicholson et al. (27) presented sixteen clinical case reports showing radiographic bone formation following treatment with the LAPIP protocol. Mailoa et al. (28) reviewed the use of the carbon dioxide and Er,Cr:YSGG lasers in the peri-implantitis surface detoxification. They concluded that there is no difference in the pocket depth reduction between the lasers use and non-surgical treatment periodontal treatment. The same issue arises for effectively studying the results of laser use in the periodontal therapy, as in the peri-implantitis treatment: different types of lasers used with varied protocols, difficult to compare the protocols.

The titanium surface of an implant adds another confounding factor to an already complex laser-tissue interaction.

Aphthous ulcer treatment is another type of a clinical laser-tissue interaction involving passing a laser over the affected ulcerated lesions. Aphthous ulcers are the most common ulcerative lesions of the oral mucosa, usually painful, and recurring. These lesions have no definitive treatment, other then palliative, and they heal in 10 to 14 days. The laser therapy has been shown effective in reducing pain and decreasing the healing time of the aphthous ulcers (29). Aggarwal et al. (29) assessed low-level laser therapy using a diode laser (810 nm) on the size and pain level of the aphthous ulcers in thirty patients. Using a diode laser resulted in shorter resolution time of the ulcers, and a complete pain relief immediately after laser application. The procedure was performed in one day, with four laser applications, each application 45 seconds in duration. Albrektson et al. (30) conducted a randomized controlled trial to measure the pain associated with recurrent aphthous ulcers in forty patients. He used a diode laser with a wavelength of 809 nm, and the laser rod was held in a direct contact to the ulcer for 80 seconds. The procedure was repeated for two consecutive days. Pain perception as recorded on the Visual Analog Scale decreased significantly by more than 50 percent. It can be concluded that a diode laser can be used to help speed the healing time of the aphthous ulcers, and decrease the pain experienced by patients.

However, there is no standardized treatment protocol, and the two studies mentioned have very different treatment regimens for the aphthous ulcers, both appearing effective.

Biopsy is a surgical term used in the case of tissue removal for pathological examination. The golden standard for biopsy is a stainless steel scalpel blade incision, which does not alter the tissues and cells surrounding the biopsy site. However, the use of a laser can present an advantage in biopsy of highly vascular oral tissues due to its hemostatic effect. The laser-tissue interaction changes the cells, so the margins of the biopsy site need to be carefully considered to obtain unaltered cells for pathological examination (8, 16). Also, the vaporizing effect of a laser naturally eliminates any tissue evidence for further examination. Seoane et al. (16) used the Er,Cr:YSGG laser for excisional biopsy of oral leukoplakia, and he concluded that this laser offers minimum amount of thermal artifacts at the surgical margins. The carbon dioxide and Nd:YAG lasers can also be used to biopsy oral leukoplakia lesions.

Bacterial disinfection in non-surgical periodontal treatment is an example of a laser-tissue interaction that targets the bacteria present in oral biofilm. Periodontitis is caused by bacteria, and the conventional non-surgical periodontal therapy strives to remove pathogenic bacteria. Moreira et al. (31) conducted a split-mouth randomized controlled trial to measure antimicrobial effect of a diode laser (670 nm) as an adjunct to scaling and root planing in patients with aggressive periodontitis. The results were significant for eliminating periodontal pathogens using a laser in addition to scaling and root planing.

MATERIAL AND METHODS

Animals

Three fresh pig mandibles purchased from the company, Sierra for Medical Science (Whittier, California), shipped fresh within 24 hours of slaughter in ice packs were utilized in this study. The mandibles were kept cool until one hour before the experiment, and then warmed to room temperature.

Incision

Six incisions, three on the facial, three on the lingual, approximately 22 mm in length, were made by an experienced operator in the keratinized oral mucosa parallel to the border of the mandible, and 3 mm below the gingival margin, with a scalpel, and the four lasers tested (Figure 1).

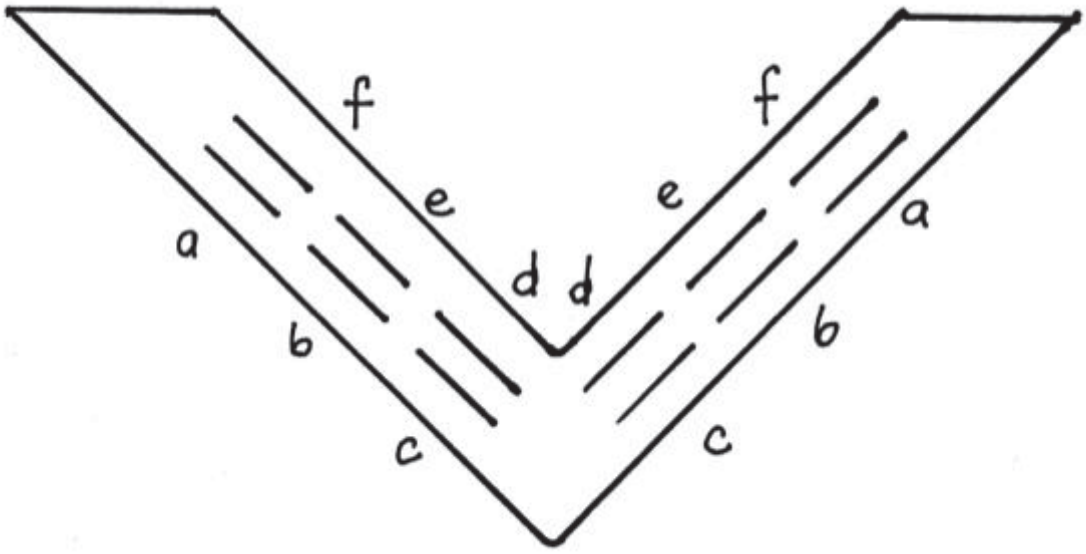

Figure 1: Diagram of the study design in pig mandible showing incision site position: a) posterior facial, b) middle facial, c) anterior facial, d) anterior lingual, e) middle lingual, f) posterior lingual.

A total of 30 incisions were made, 6 incisions with scalpel and four lasers tested. Immediately following incisions, the tissue specimens were prepared for histological examination by fixing them in formalin, sectioned at 100 microns, and stained with hematoxylin and eosin to assess incision depth, incision width, lateral coagulation, and deep coagulation. There were four sections taken from the middle of the incision zone, for a total of 120 slides for histological examination (5). Each sample was numbered to allow for the histological examiner to be blinded to the treatment modality. Each incision was timed with a stopwatch by another person to determine the time of incisions in seconds. Afterwards, digital photographs were taken with Nikon D7000 digital camera to record the clinical appearance of the incisions. The stainless steel scalpel blade incision was made to the bone, while the laser incisions followed the manufacturers' instructions for each laser type. A stainless steel scalpel blade, Bard Parker No 15, was used to perform control incisions.

The test incisions were performed using the carbon dioxide laser, diode laser, Nd:YAG, and Er,Cr:YSGG lasers, following the manufacturers' instructions for the soft tissue incision. The settings for each type of laser utilized are listed below.

Laser Settings

1. Carbon dioxide laser, Luxar 20 (LX-20, Luxar Corp., Bothell, WA) 5 Watts, was used in a non-contact, continuous wave mode, with 0.8 mm tip diameter. The incisions were performed 5 mm from the tissue.

2.Er,Cr:YSGG laser, Waterlase iPlus (Biolase Technology, Inc., Irvine, California) was used in a 3D setting: 2 Watts, S mode, 30 pps, 10% water, 30% air.
Incisions were made with MZ6 tip, 9 mm length in a gold handpiece in a contact, pulse mode.

3. Diode laser, Zap (Zap Lasers, Pleasant Hill, California) 808 nm was used at 1.2 Watts, with an articulating paper initiated fiberoptic flexible tip in a non-contact, continuous wave mode.

4. Nd:YAG laser, Periolase MVP-7 (Millenium Dental Technologies, Inc., Cerritos, California) 4 Watts, in contact mode, 10 microseconds, 20 Hz, tip diameter 320 microns.

Protocol

The three pig mandibles were randomly assigned. Treatment groups were given a number to blind the histological examination:

Jaw A: carbon dioxide laser and Er,Cr:YSGG laser

Jaw B: scalpel and diode laser

Jaw C: Nd:YAG laser

120 slides were made for the histological examination, however due to sampling and processing errors, such as tearing and folding, 85 slides had to be excluded.

Number of included slides in each group: scalpel 3, carbon dioxide laser 9, Er,Cr:YSGG laser 9, diode laser 6, Nd:YAG laser 8.

35 slides were read and measured by a calibrated examiner using a microscope (Olympus BX51) with 4x and 10x objective. Each of the slides had between 2 and 5 measurements of each parameter recorded in microns, and the full length of each specimen was examined, with the measurements being evenly spaced along the incision. The number of measurements made per slide depended on the examiners confidence in the clarity of measuring histological changes in soft tissue with each treatment. The examiner was calibrated prior by examining, measuring, and recording every slide on 3 separate occasions, a week apart each time, until the measurements became consistent. The 4^{th} time measurements were recorded for all the readable slides, and constitute the raw data.

The parameters tested were measured using Image-Pro Plus 6.0 software (Silver Spring, Maryland). Each slide was measured for the depth and width of incision as well as deep and lateral coagulation adjacent to tissue ablation zone (Figure 2), and varying number of observations was recorded, ranging from 2 to 5 per each parameter measured. Tissue necrosis and damage was assessed histologically by looking microscopically at the loss of intercellular boundries, intracellular vacuolations, and changes in staining reaction (8).

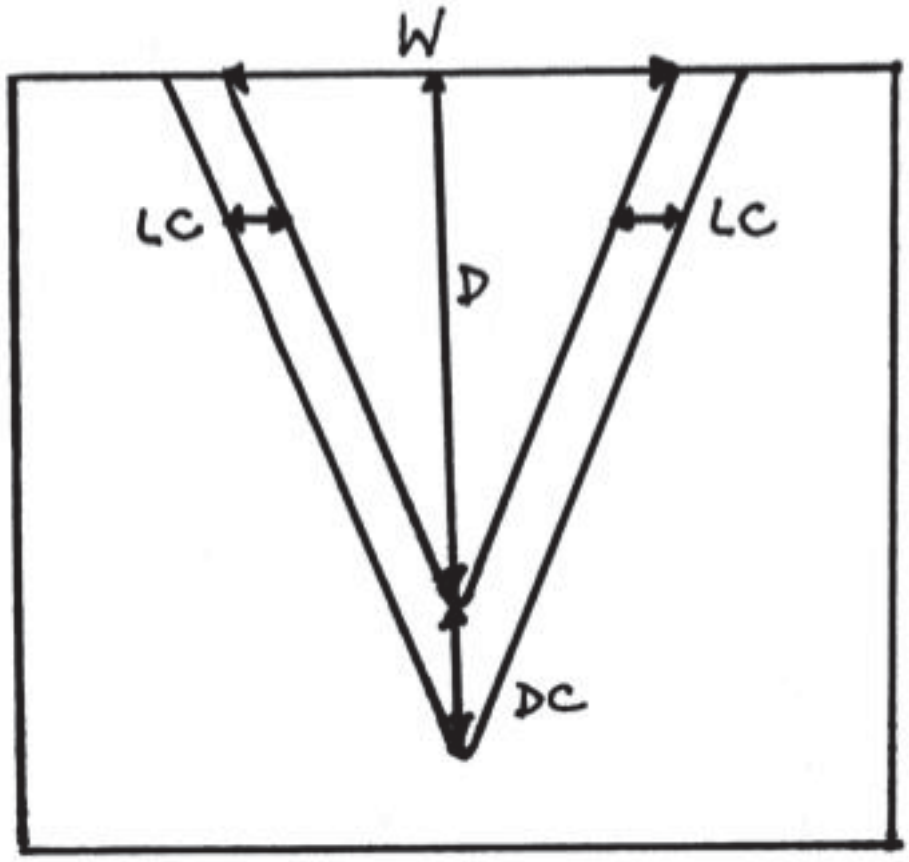

Figure 2: Diagram of the zones of effect measured microscopically: W) width of incision, D) depth of incision, LC) lateral coagulation, DC) depth of coagulation.

Statistical Analysis

Power analysis was performed prior to conducting the experiment. The assumptions made: α = 0.05 (two-sided), power = 0.9 yielded the estimated sample size n = 22 per parameter tested. A one-way analysis of variance (ANOVA) was used to test for the differences between and within the groups, with posthoc either Scheffe or Bonferroni correction, whichever generated a P value.

The P value was set at <0.05. The STATA 11.1 software (Stata Corp, College Station, TX) was used for the statistical analysis.

RESULTS

Macroscopic Analysis

A macroscopic analysis of the five methods showed that the scalpel made the least incision while the lasers would leave a scar-like incision related to tissue ablation and coagulation (Figure 3).

Carbon dioxide laser

Diode laser

Er,Cr:YSGG laser

Nd:YAG laser

Stainless steel scalpel blade

Figure 3: Digital clinical photographs of one representative incision with each treatment

Histology

Photomicrographs of histology of one representative incision per treatment modality, 4x magnification, with incision site position as in Figure 1, hematoxylin and eosin staining.

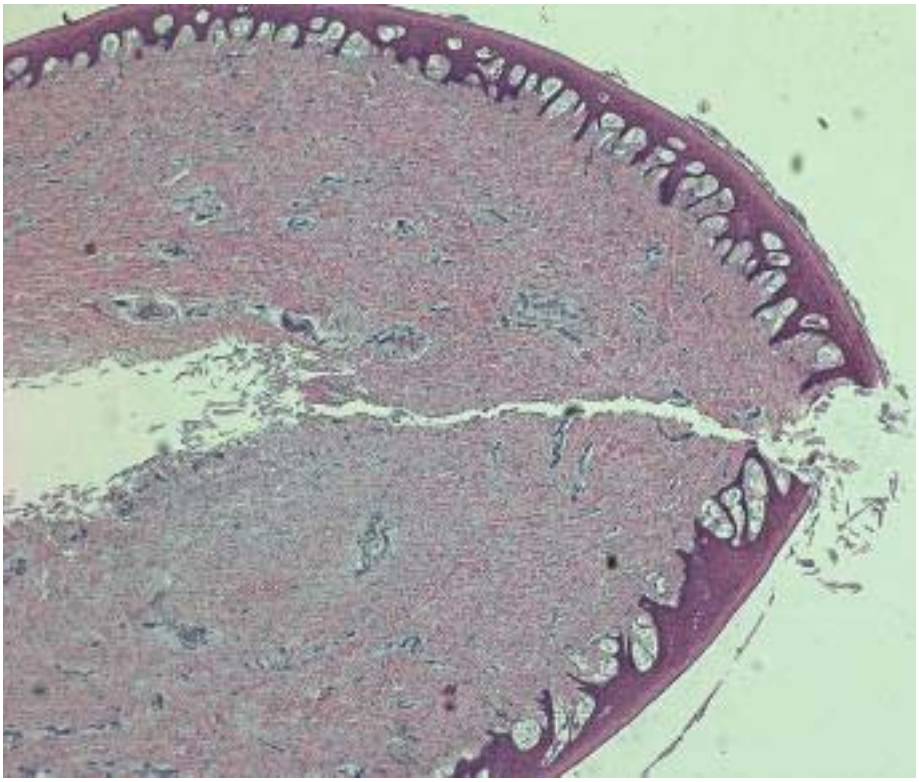

Figure 4: Effect of the stainless steel scalpel blade incision made to the bone of the pig mandible soft tissue in the posterior facial position. Notice no coagulation effect, just tissue separation.

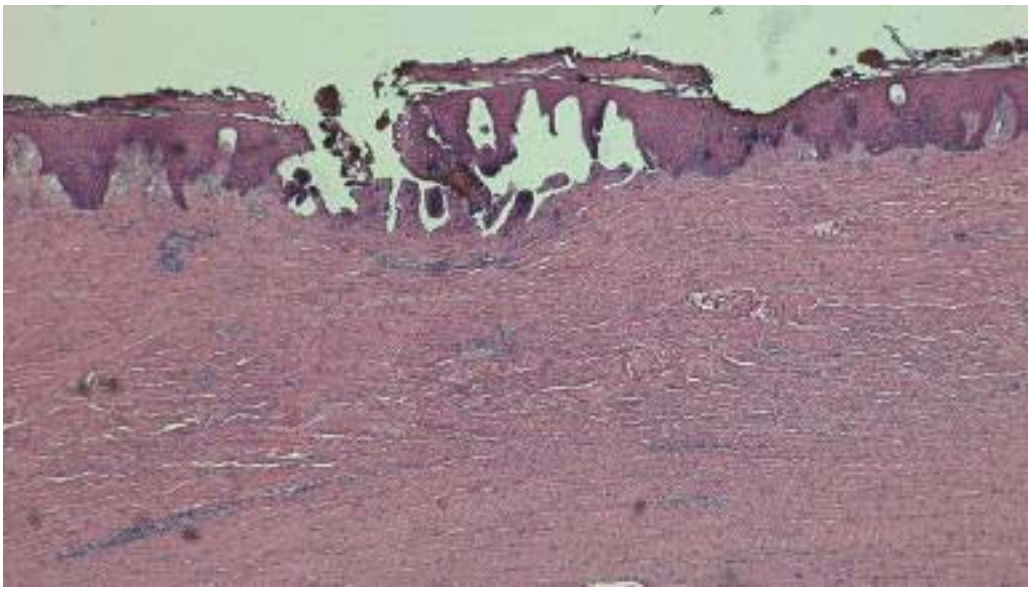

Figure 5: Soft tissue incision made with the carbon dioxide laser in the middle lingual position. Notice wide spread surface carbonization, but relatively shallow depth of coagulation.

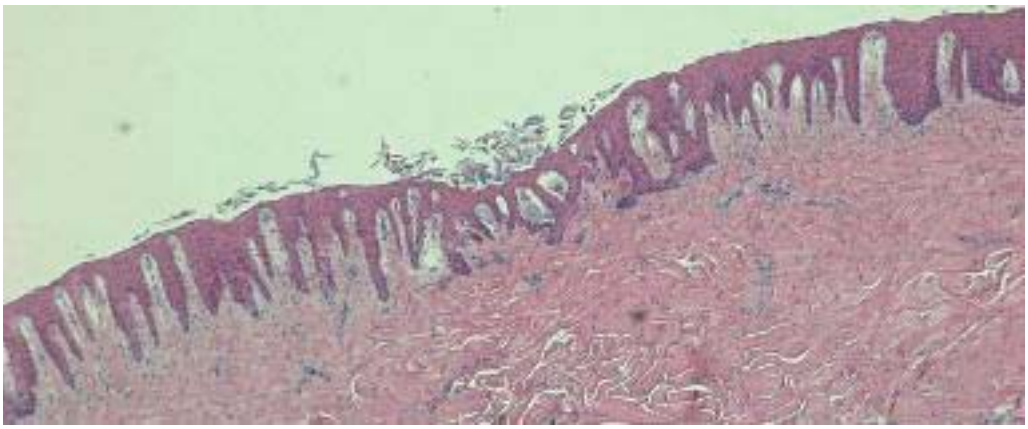

Figure 6: Er,Cr:YSGG laser soft tissue incision in pig mandible in the anterior facial position. Notice shallow depth of coagulation, and wider lateral coagulation.

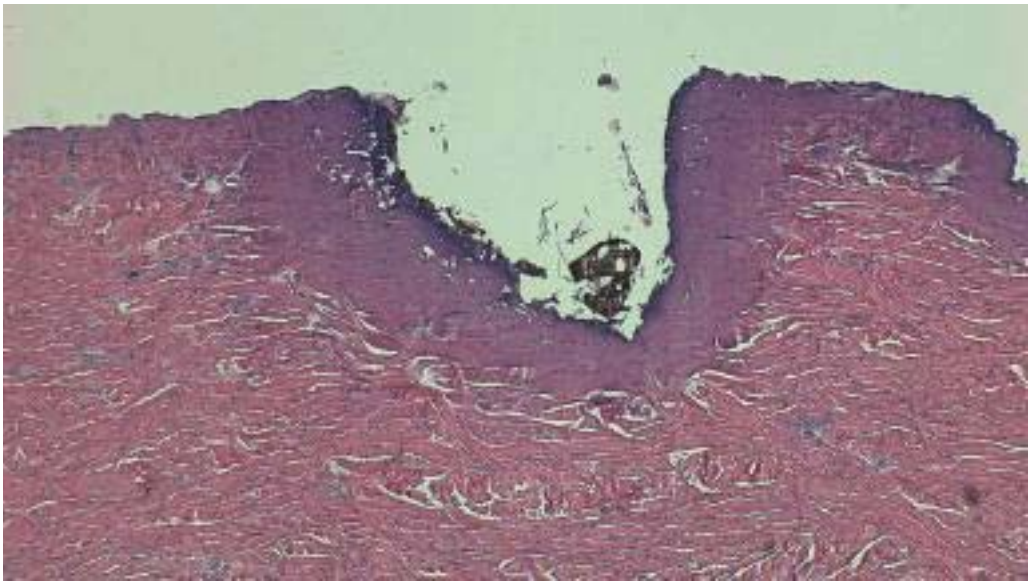

Figure 7: Soft tissue incision made with the diode laser in the anterior lingual position in pig mandible. Notice large ablation cavity with extensive deep and lateral coagulation.

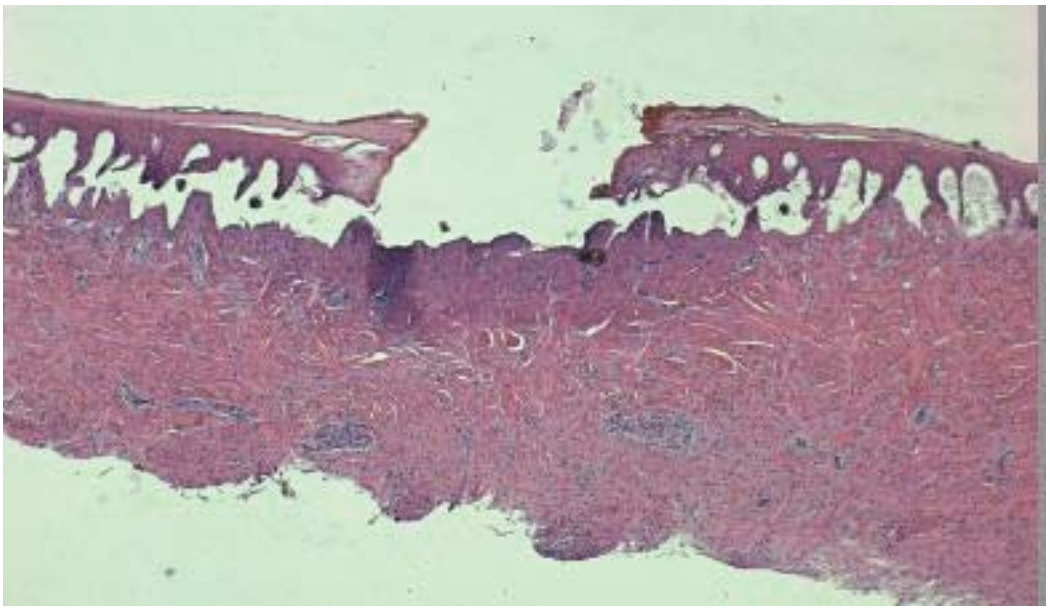

Figure 8: Nd:YAG laser incision in the middle lingual position in pig mandible. Notice the separation of epithelium from the connective tissue and depth of coagulation.

Photomicrographs of histology of one representative treatment, 10x magnification. Hematoxylin and eosin staining. The histological slides of 10x magnification are not the same as the previously shown specimens for 4x magnification, with the exception of the scalpel incision slide, which is the same specimen under 4x and 10x magnification.

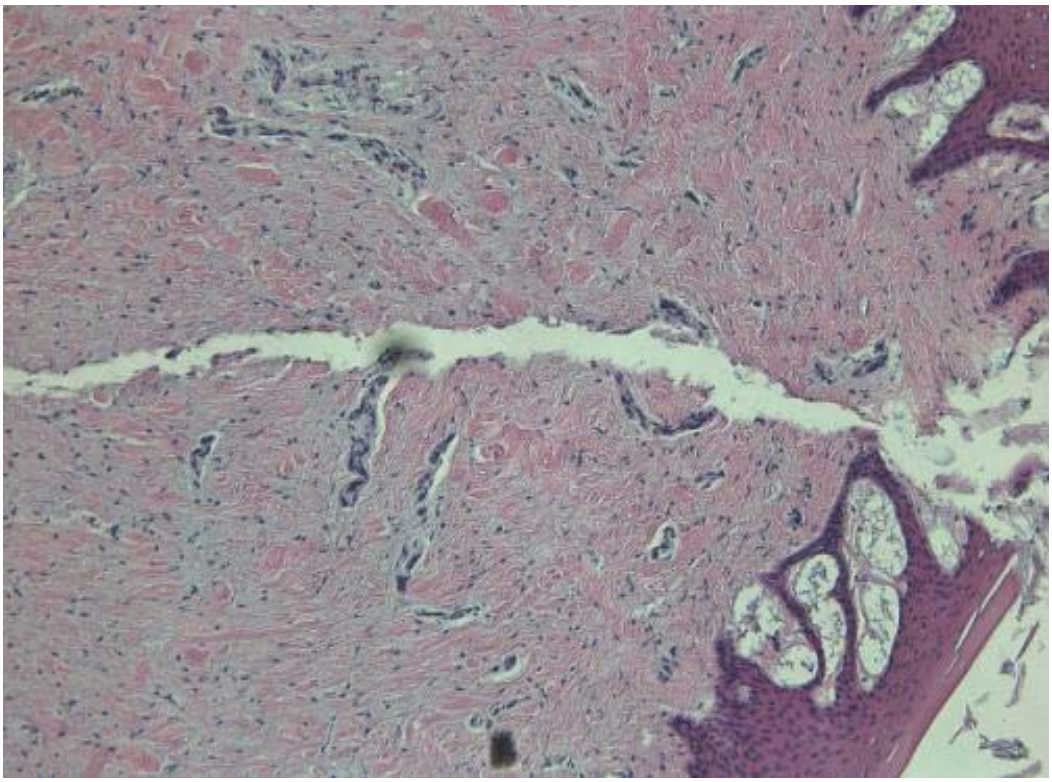

Figure 9: Effect on the soft tissue of the pig mandible using the stainless steel scalpel blade. Notice no coagulation but just separation of tissue.

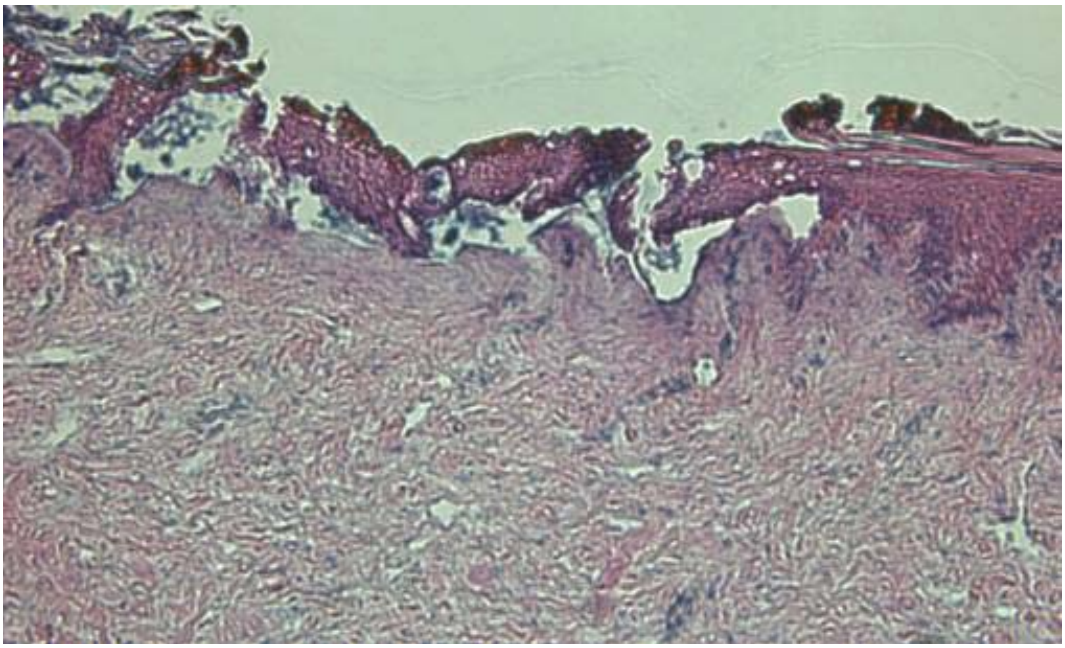

Figure 10: Histological slide of the incision made with the carbon dioxide laser. Notice extensive surface carbonization.

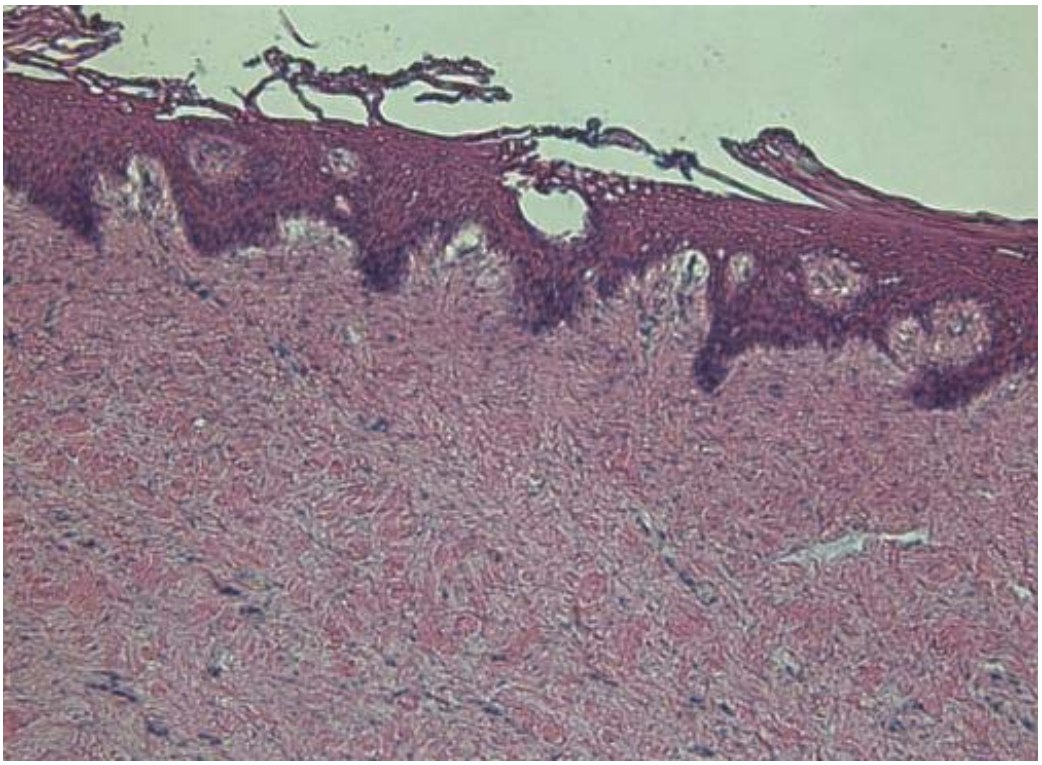

Figure 11: Histological slide of the incision made by the Er,Cr:YSGG laser. Notice largely intact surface epithelium.

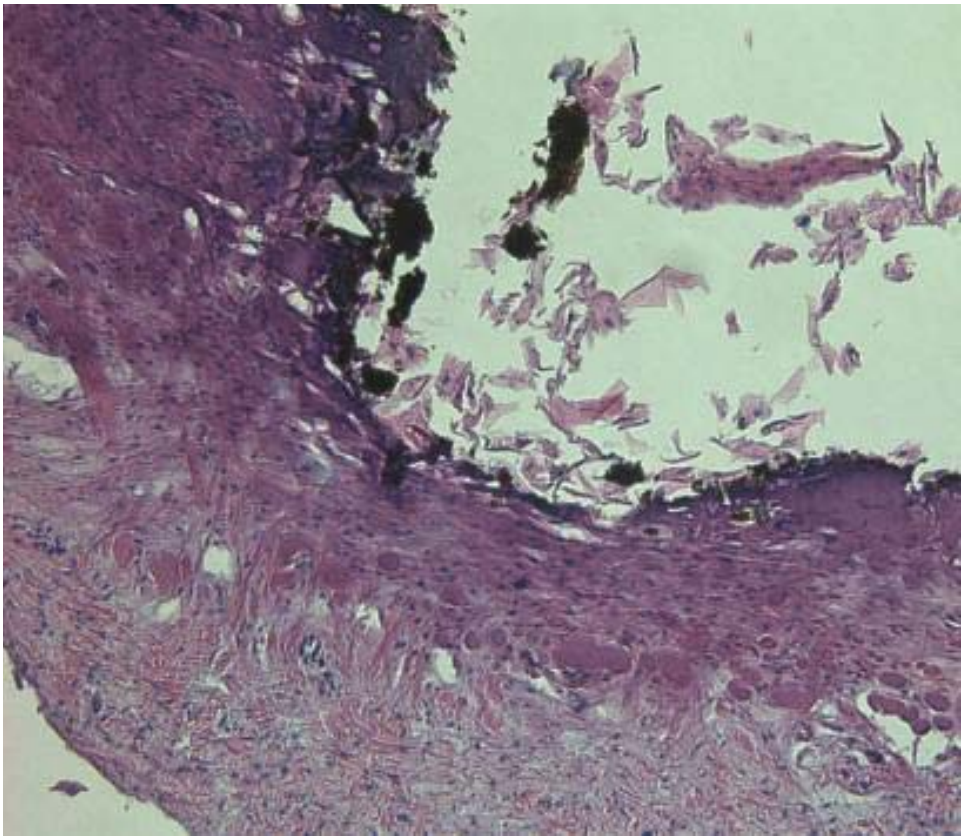

Figure 12: Histological slide of the incision made by the diode laser. Notice deep tissue necrosis and carbonization, and extensive lateral and deep coagulation.

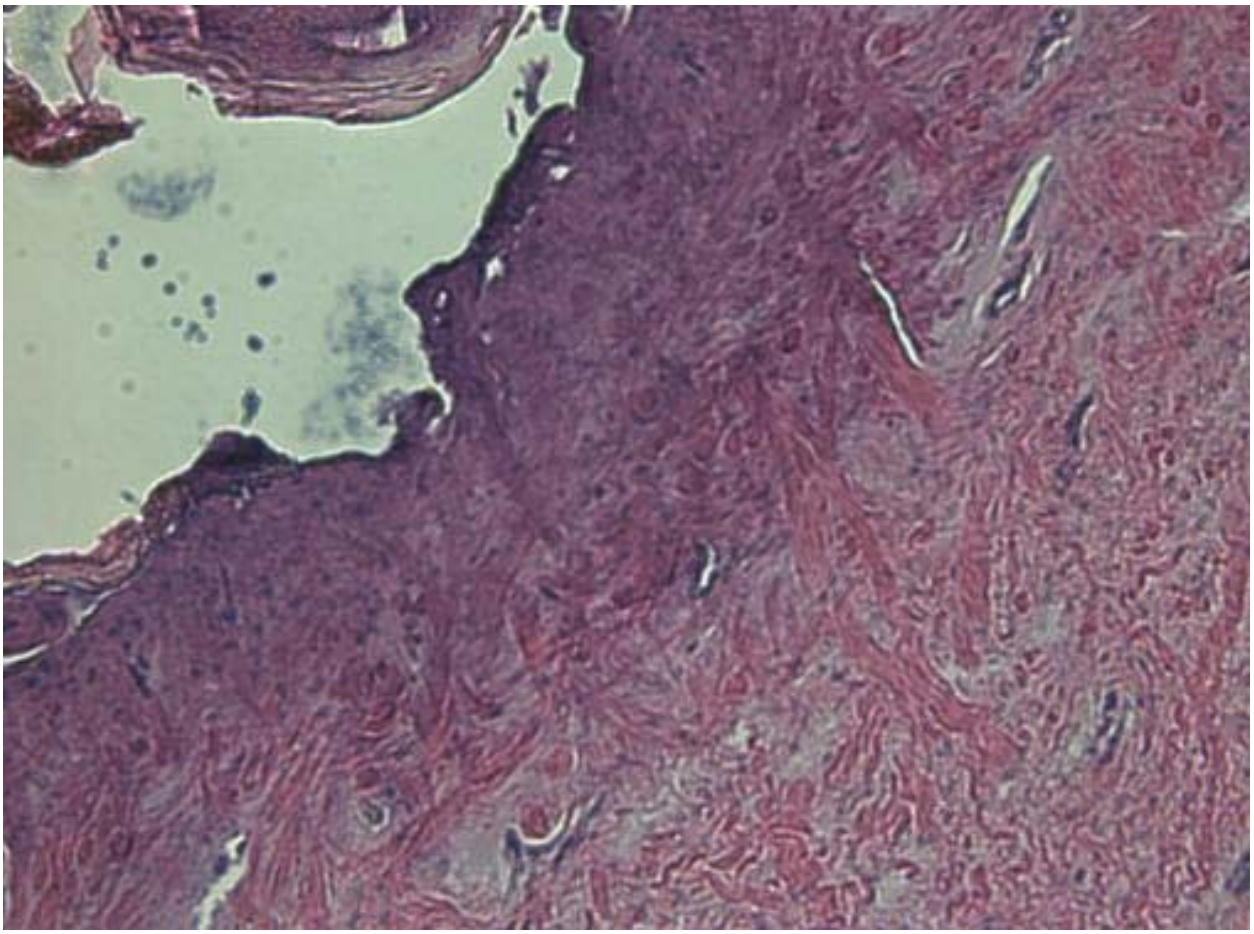

Figure 13: Histological slide of an incision made by the Nd:YAG laser. Notice extensive coagulation past the tissue ablation zone.

Width of Incision

The width of incision measured the tissue necrosis zone in its horizontal component (Figure 2). Each treatment modality had multiple measurements taken of the each readable slide under 10x magnification, after examining the full length of a slide under 4x magnification.

For example, the width of incision for the scalpel 3 readable slides was measured 15 times, 5 times per slide, producing 15 observations for the statistical analysis. The carbon dioxide laser width of incision was measured 28 times, between 2 and 5 observations per slide. The numbers of observations per treatment group, with their mean and standard deviations, are summarized in Table 1.

The comparison of the five different approaches of the width of incision showed that the scalpel made the least width as compared to the four lasers (Figure 14). ANOVA with posthoc Bonferroni and Scheffe correction indicated that the carbon dioxide laser, the Er,Cr:YSGG laser, and the diode laser incisions were all significantly different from the Nd:YAG laser incision in the soft tissue of pig mandible. The carbon dioxide laser was not significantly different from the Er,CR:YSGG and the diode laser. The Er,Cr:YSGG laser was not significantly different from both the carbon dioxide and the diode lasers. Analysis of the mean and 1 standard deviation indicated the same relationship with the scalpel doing the least incision width (mean 50.60 microns), and the Nd:YAG laser doing the most width of incision (mean 1331.31 microns). The carbon dioxide laser with a mean 573.62 microns, and the Er,Cr:YSGG laser with a mean 669.71 microns made the least wide incisions from the laser group, followed by the diode laser with a mean 852.52 microns (Table 1), (Figure 15).

Table 1: Width of Incision

Variable	Obs	Mean	Std. Dev.	Min	Max
Scalpel	15	50.60733	18.5255	17.02	86.16
Laser1	28	573.6271	482.1939	95.65	1838.61
Laser2	28	669.715	394.1349	261.28	1711.39
Laser3	21	852.521	430.7994	206.02	1750.78
Laser4	27	1331.312	595.8052	355.52	2483.66

Laser 1: carbon dioxide; Laser 2: Er,Cr:YSGG; Laser 3: diode; Laser 4: Nd:YAG

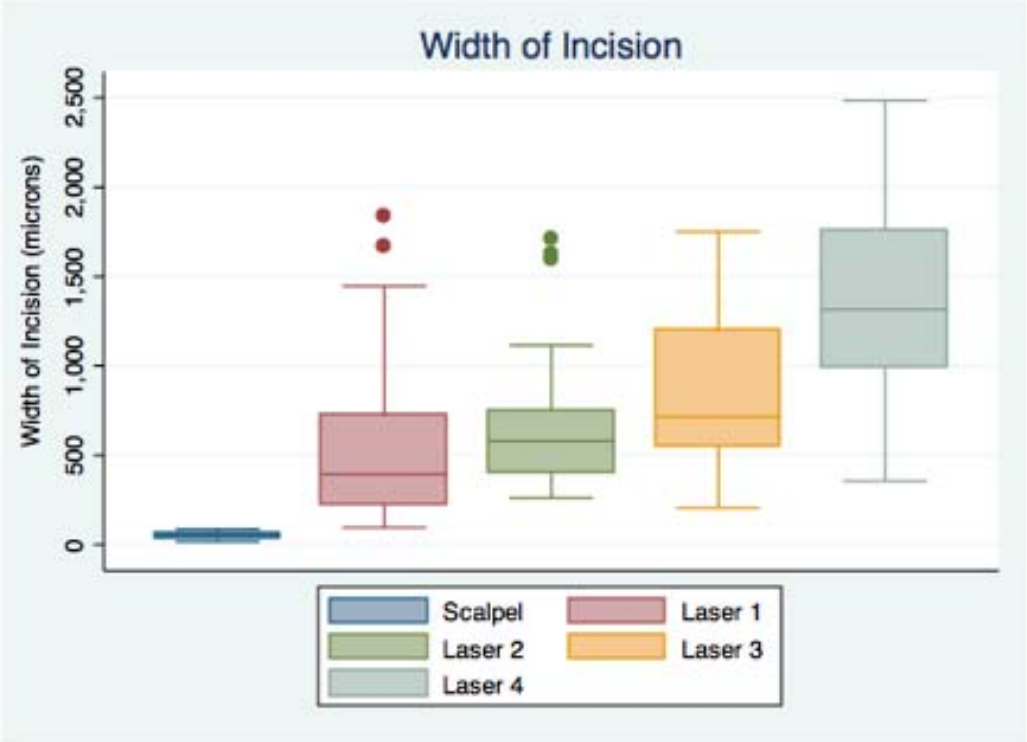

Figure 14: The width of incision is shown as a boxplot indicating the median, the 25% percentile (lower box border), the 75% of the percentile (upper box border), and the range (outer lines). Laser 1: carbon dioxide, Laser 2: Er,Cr:YSGG, Laser 3: diode; Laser 4: Nd:YAG. Notice that the width of the incision is smallest with the scalpel.

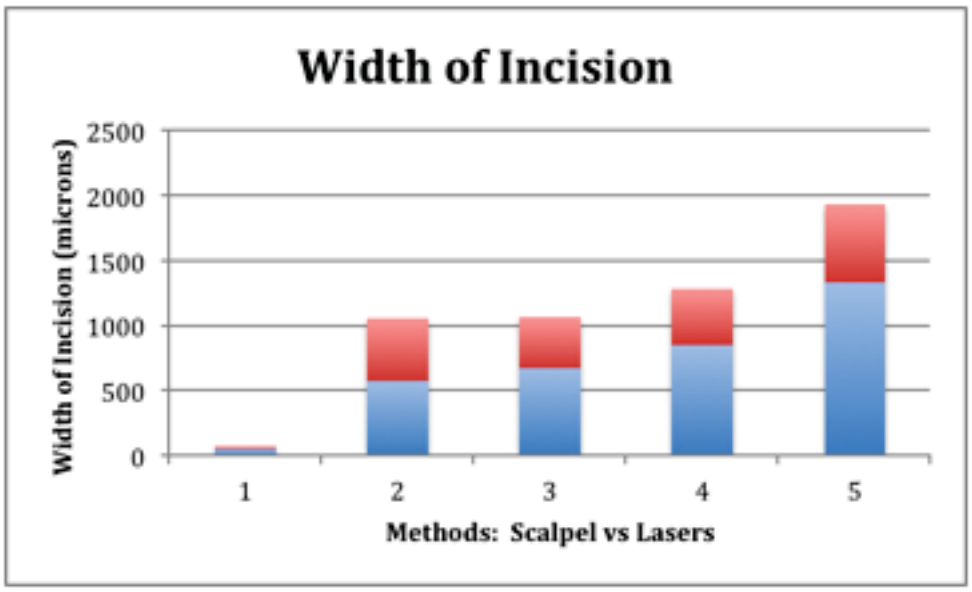

Figure 15: Mean and 1 SD of the five methods and the width of incision made by each method. The scalpel made the least width of incision while the Nd:YAG laser demonstrated the most width. 1) scalpel; 2) carbon dioxide laser; 3) Er,Cr:YSGG laser; 4) diode laser; 5) Nd:YAG laser

Depth of Incision

The depth of incision measured the tissue necrosis zone in its vertical component (Figure 2). Comparison among the five approaches to incise soft tissue showed that scalpel induced the deepest incision compared to the lasers (Figure 16). A one way ANOVA with posttest Scheffe or Bonferroni to obtain the P value set at $P<0.05$ shows that the scalpel does the deepest incision, statistically significantly different than the four lasers, with a $P<0.05$. The carbon dioxide laser and the Er,Cr:YSGG laser depth of incision was significantly different than the diode and Nd:YAG lasers.

There was no statistical difference between the diode and Nd:YAG lasers depth of incision. Evaluating the mean and 1 SD showed that the scalpel induced average depth of 1406.365 microns compared to the four lasers. The least depth was done by the Er,Cr:YSGG laser (mean 153.74 microns), followed by the carbon dioxide laser (mean 222.10 microns) (Figure 17), (Table 2).

Table 2: Depth of Incision

Variable	Obs	Mean	Std. Dev.	Min	Max
scalpel	6	1406.365	96.26716	1239.56	1476.6
laser1	26	222.1008	96.45433	47	357.55
laser2	29	153.7469	77.79324	47.58	334.66
laser3	20	517.766	276.2086	115.76	900.86
laser4	24	423.485	216.152	93.71	803.85

Legend for Table 2 and Figure 16: Laser 1: carbon dioxide; Laser 2: Er,Cr:YSGG; Laser 3: diode; Laser 4: Nd:YAG. Obs=number of measurements per readable slide.

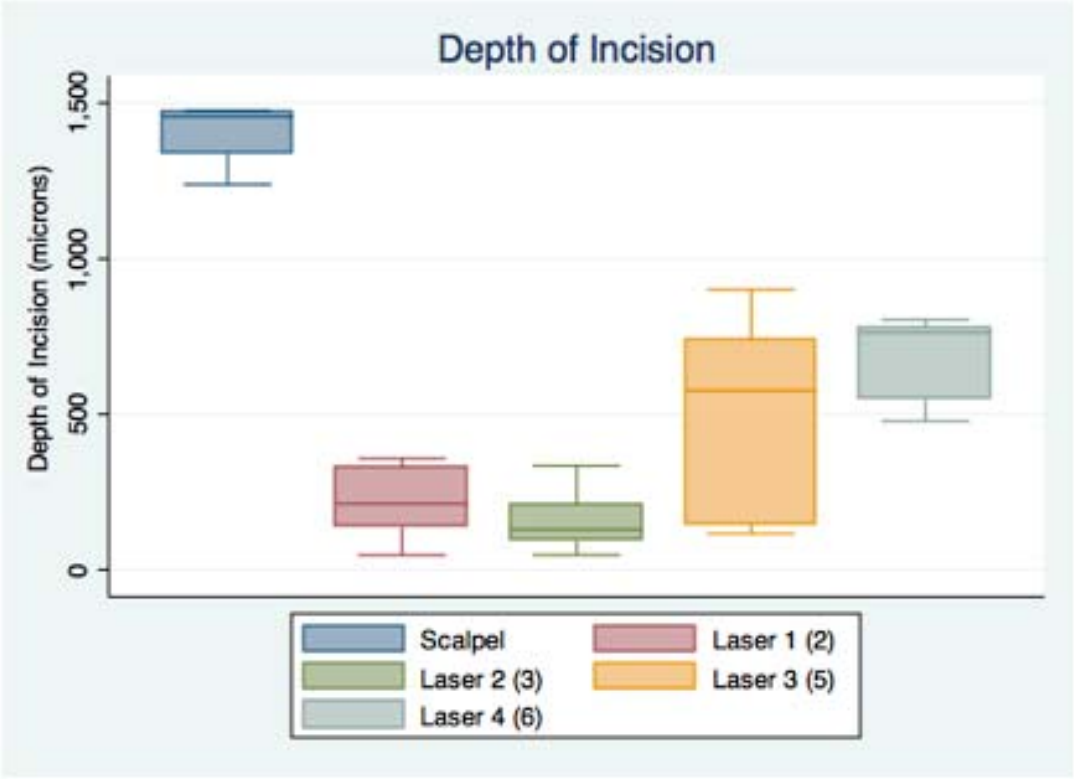

Figure 16: The depth of the incision is shown as a boxplot indicating the median, the 25% percentile (lower box border), the 75% of the percentile (upper box border), and the range (outer lines). Notice that the scalpel induced the most depth of incision.

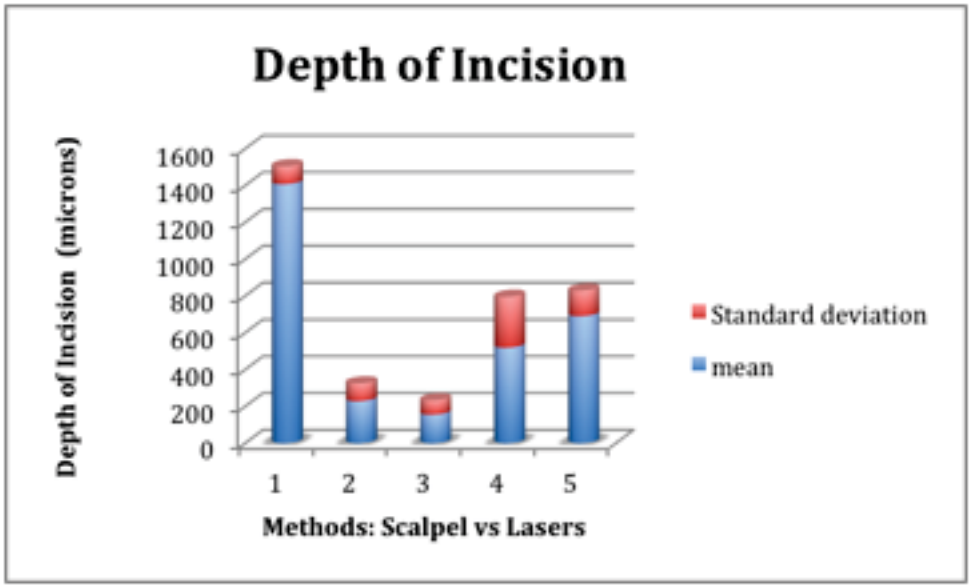

Figure 17: The mean and 1 SD of the depth of incision shows that the scalpel induced the deepest incision while the carbon dioxide and Er,Cr:YSGG lasers did the least depth. 1) scalpel; 2) carbon dioxide; 3) Er,Cr:YSGG; 4) diode; 5) Nd:YAG.

Lateral Coagulation

Lateral coagulation measured soft tissue changes at the lateral border of the incision (Figure 2). There were only 3 observations recorded for the scalpel incision, because histologically there was no lateral coagulation noted on the 3 slides available. The lateral coagulation for each laser varied from wide at the surface epithelium border and narrowing at the sides of the ablation cavity, as the full length of the slide was examined under 4x magnification first. Lateral coagulation on the right and left side of the ablation zone was measured, as well as the lateral coagulation on the epithelial surface.

Thus, the lateral coagulation was measured from the widest, surface points, then tapering laterally on either side of the ablation zone, generating 5 observations per readable slide for statistical analysis. Comparison of the five methods for lateral coagulation showed that the scalpel induced none, while the four lasers gave similar results (Figure 18), with the diode laser showing the widest variation in the width of coagulation. One way ANOVA with $P<0.05$ and a posthoc test of either Scheffe or Bonferroni that give the P test showed that the scalpel created statistically significantly less lateral coagulation compared only to the carbon dioxide laser. The Er,Cr:YSGG laser was statistically different only from the diode laser, and the diode laser had much more variability. Comparing the five methods by their mean and 1 SD showed similar results (Figure 19). The scalpel had no lateral coagulation, while the diode laser had the most lateral coagulation with a mean 1138.17 microns, followed by the carbon dioxide laser with a mean 1092.91 microns. The Er,Cr:YSGG laser showed the least lateral coagulation in the laser group with a mean 563.77 microns (Table 3).

Table 3: Lateral Coagulation. Laser 1: carbon dioxide, Laser 2: Er,Cr:YSGG, Laser 3: diode, Laser 4: Nd:YAG

Variable	Obs	Mean	Std. Dev.	Min	Max
scalpel	3	1	0	1	1
laser1	31	1092.915	561.8422	482.37	2385.11
laser2	36	563.7725	222.1057	155.83	1021.32
laser3	19	1138.177	807.5825	278.83	2466.27
laser4	31	774.5958	683.582	158.99	2657.44

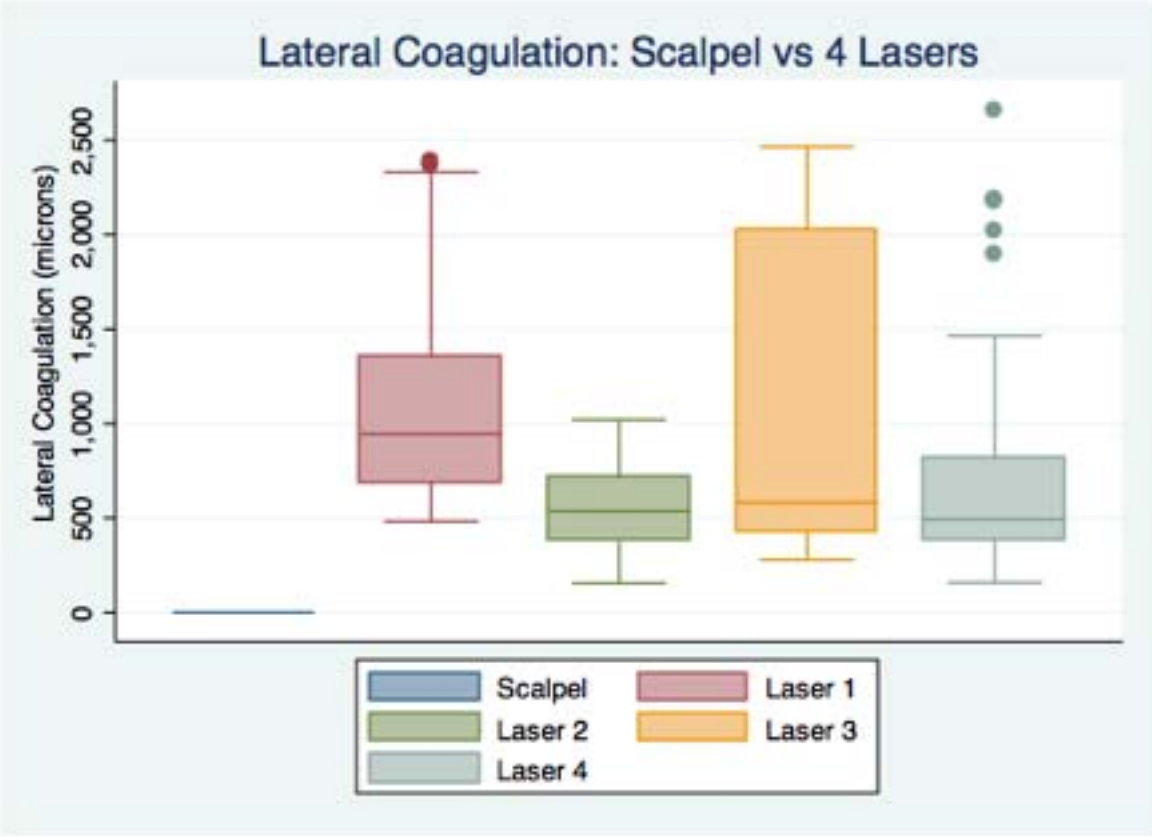

Figure 18: Extent of lateral coagulation among the five approaches indicated the scalpel induced none while all four lasers induced similar lateral coagulation.

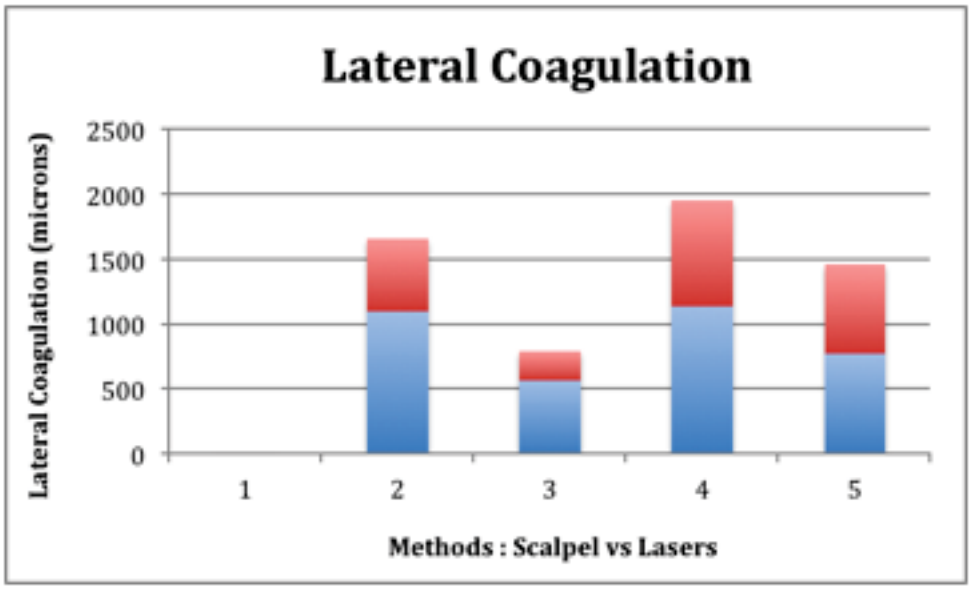

Figure 19: Mean and 1 SD of the extent of lateral coagulation in which the scalpel (1) induced none but the Er,Cr:YSGG laser (3) and the Nd:YAG (5) laser induced the least.

Depth of Coagulation

The depth of coagulation was measured at the apical component of an incision (Figure 2). The scalpel incision had no deep coagulation zone, and the incision was made to the bone, therefore only one observation was recorded for each of the 3 slides. The depth of coagulation was compared among the five methods, with the scalpel inducing no deep coagulation, but with the diode laser and the Nd:YAG laser inducing the most depth of coagulation (Figure 20). The mean and 1 SD indicated that depth of coagulation increased with the lasers (Figure 21).

The Nd:YAG laser had the most depth of coagulation with a mean 377.90 microns, and the diode laser following with a mean 281.51 microns. The carbon dioxide laser had the least depth of coagulation with a mean 148.11 microns, followed by the Er,Cr:YSGG laser with a mean 168.92 microns (Table 4). The scalpel is statistically different to each laser, P value 0.085 with the carbon dioxide laser, P value 0.031 for the Er,Cr:YSGG laser. Both the carbon dioxide and the Er,Cr:YSGG lasers are significantly different from the diode and the Nd:YAG lasers. The diode laser is significantly different from the Nd:YAG laser.

Table 4: Depth of Coagulation. Laser 1: carbon dioxide; Laser 2: Er,CR:YSGG; Laser 3: diode; Laser 4: Nd:YAG

Variable	Obs	Mean	Std. Dev.	Min	Max
scalpel	3	1	0	1	1
laser1	31	148.1187	43.56153	88.76	274.46
laser2	36	168.9269	66.18516	73.15	319.08
laser3	21	281.5171	92.74183	155.65	475.72
laser4	30	377.9083	122.5965	184.21	607.59

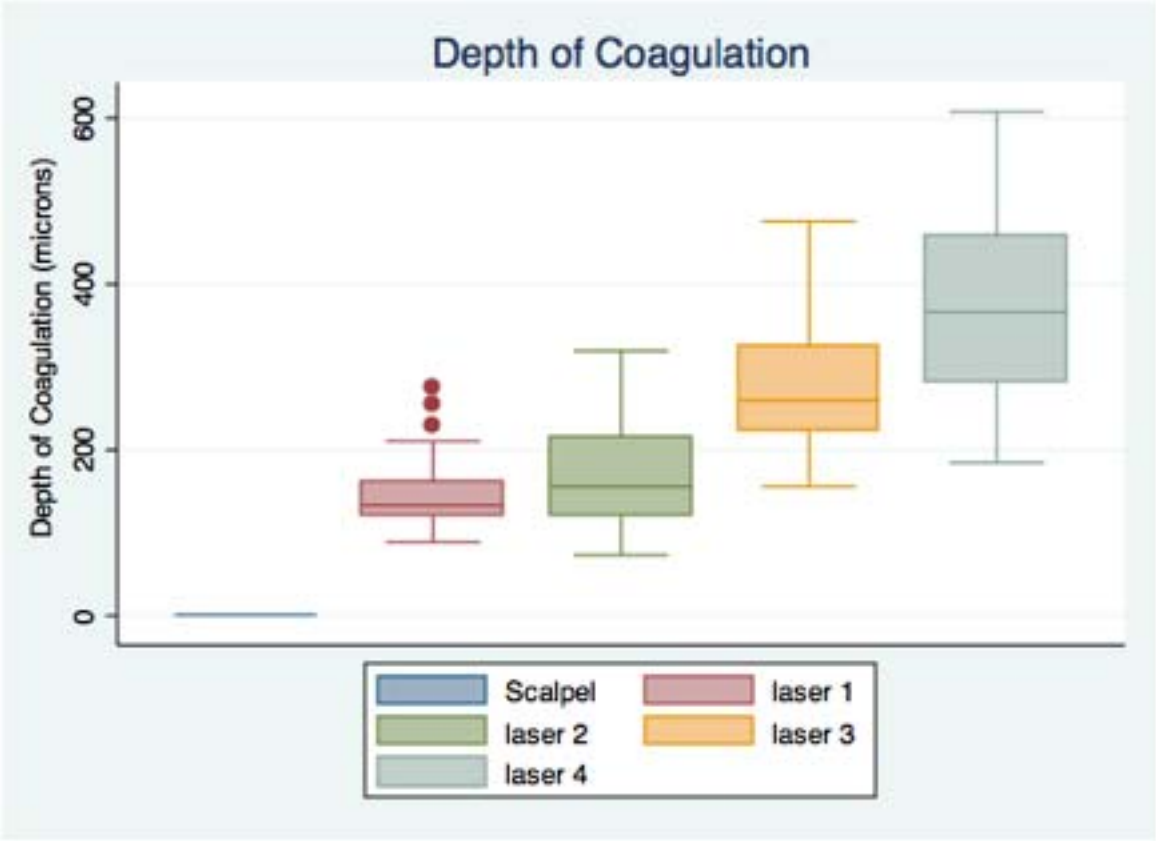

Figure 20: Boxplots indicating the depth of the coagulation with the scalpel inducing no coagulation while the diode laser and Nd:YAG laser induced the greatest depth. Laser 1: carbon dioxide, Laser 2: Er,Cr:YSGG, Laser 3: diode, Laser 4: Nd:YAG.

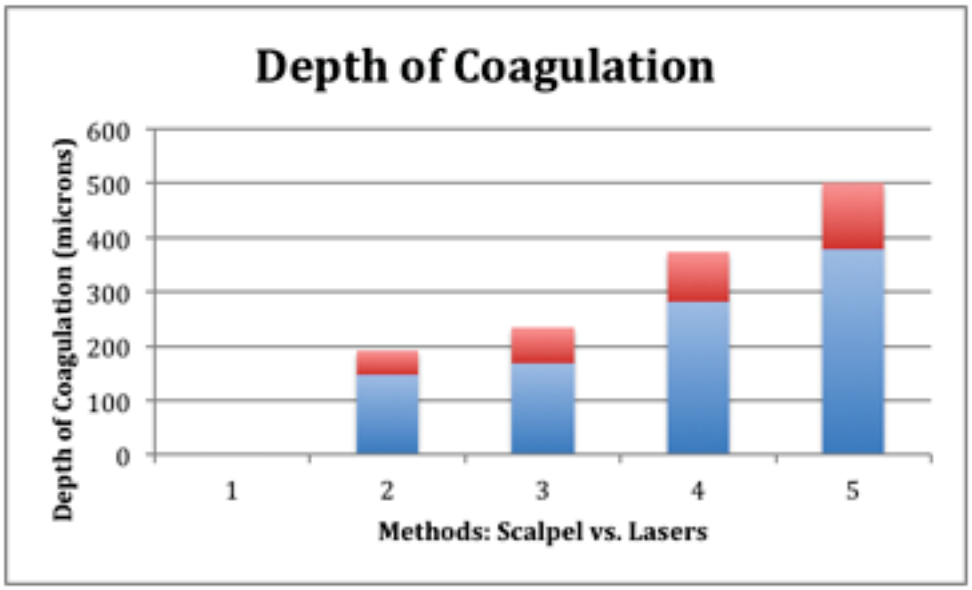

Figure 21: The depth of coagulation as induced by the five different methods indicates the scalpel does not induce any deep coagulation while lasers, diode and Nd:YAG induce the most. 1) scalpel; 2) carbon dioxide laser; 3) Er,Cr:YSGG laser; 4) diode laser; 5) Nd:YAG laser.

Other Observations

Comparison among the five methods showed the time to complete the same type of incision was least with the scalpel, and increased with the lasers (Figure 22). No statistical analysis for the time of incision was performed due to small sample. The incisions were made on the facial and lingual of a pig mandible, because the keratinized gingiva in a pig resemble human gingiva. Clinically, during the experiment, there was a difference in the way lasers interacted with the tissue based on the thickness of the gingiva, and its position in the pig jaw.

Based on our clinical observation, the thickest keratinized gingiva was located in the anterior facial and lingual sites, and the thinnest was in the posterior lingual site. However, due to small sample size, and exclusion of slides, we were unable to perform statistical analysis of the effect of each laser and scalpel on the position of tissue site within pig jaw. This was reflected in the time for incision measurement: the scalpel cut was the fastest regardless of how thick the gingival tissue was, from 3.5 to 1.8 seconds. The thicker the gingival tissue, the longer it took to make laser incisions due to laser-tissue interaction. The longest time for incision was with the Nd:YAG laser. In the thickest gingival region in the anterior lingual site, it took the Nd:YAG laser 34.9 seconds to make a 22 mm incision, and in the anterior facial site it was 34.7 seconds to make a similar length incision. The second fastest incision was made with the carbon dioxide laser, ranging from 5.9 to 4.5 seconds. The Er,Cr:YSGG laser incised soft tissue in the time range from 16.6 to 8.1 seconds. The diode laser made an incision in the time ranging from 18.3 to 9.9 seconds.

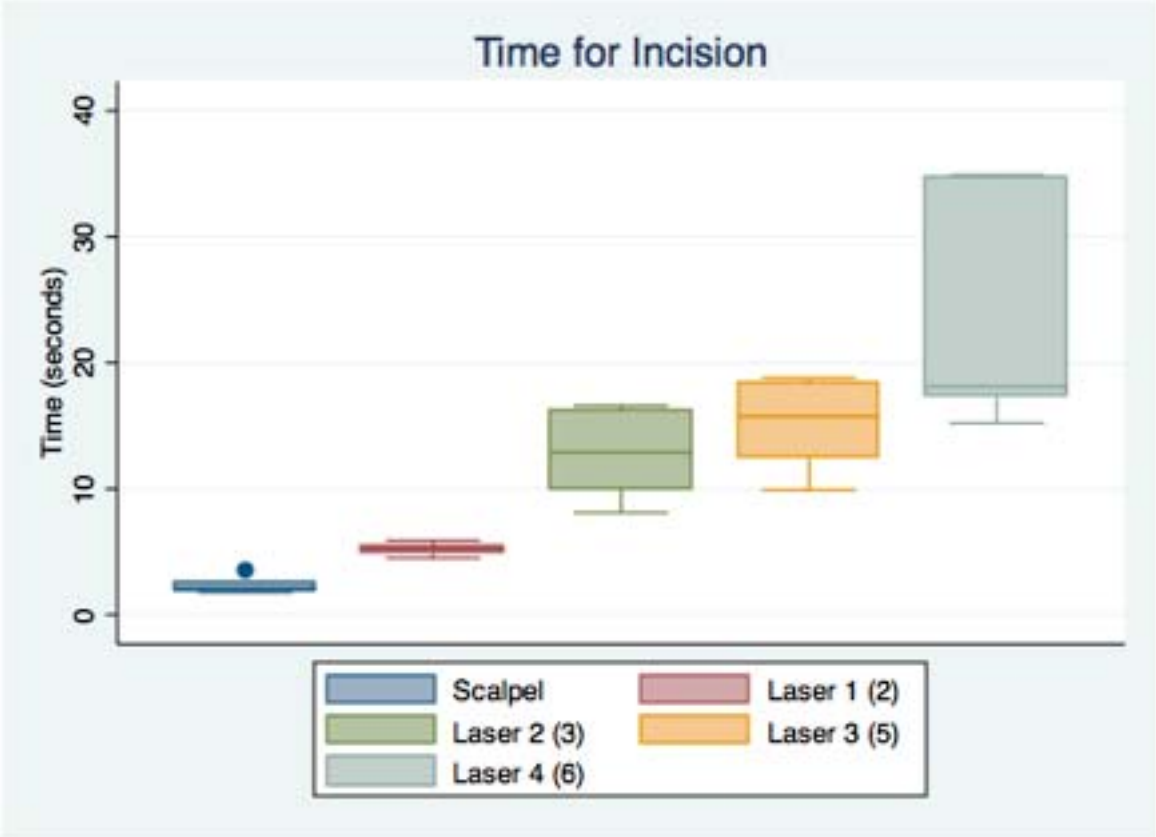

Figure 22: Time to induce a similar length incision was compared among the five methods with the scalpel being the most rapid. Laser 1: carbon dioxide; Laser 2: Er,Cr:YSGG; Laser 3: diode; Laser 4: Nd:YAG.

DISCUSSION

The five different histological measurements indicated that the scalpel caused no lateral or deep coagulation zone in the soft tissue during the incision. There was also a difference among the four lasers. Pig mandible was chosen for the study, because of the similarities with human oral soft tissues.

Limitations in the study were the small number of qualified samples, which did not allow a large sample for statistical analysis, but measurements did provide a relatively clear picture of changes. Other studies indicate that there may not be a need to have large numbers of samples due to the accurate calibration of the four lasers, and the known properties of the stainless steel scalpel blade. Vanderhobli et al. (5) discusses that multiple incisions of the same parameter do not provide any additional information, and are costly to examine histologically. Another limitation of the study was wide standard deviations. Therefore, initial statistical analysis was based on nonparametric data, and the Kruskal-Wallis test was used. However, the Kruskal-Wallis gave only a very broad picture, and it did not allow for comparison within the groups.

Our study utilized the experimental design by Wilder-Smith et al. (4) with the study design, and the histologic measurements of incision depth, incision width, tissue damage depth and width. We expanded our study to include not only examination of soft tissue incisions by the carbon dioxide laser, but added diode laser, Nd:YAG and Er.Cr:YSGG lasers, and the stainless steel scalpel blade. Wilder-Smith et al. tested three different settings of the carbon dioxide laser (1 W, 4 W, and 12 W), and three different brands of the carbon dioxide laser. Our study used each laser according to each laser manufacturers' instructions for soft tissue incisions. In our opinion, this approach resembles more an everyday laser use in a clinical situation.

We used the carbon dioxide laser with 5 W setting, and our measurements for the width of incision (our mean 573.62 versus 590 microns), and the depth of incision (our mean 222.10 versus 161 microns) are very similar to the ones presented by the Wilder-Smith study for the 4 W carbon dioxide laser, Luxar Corp. brand. On the other hand, our measurements of the lateral and deep coagulation vary largely. Our lateral coagulation mean for 5 W carbon dioxide laser was 1092.91 microns versus 17.7 microns for 4 W, 33.9 microns for a 12 W carbon dioxide laser. Our depth of coagulation measurement mean was 148.11 microns versus 13.2 microns for 4 W, and 21.2 microns for a 12 W carbon dioxide laser. The possible reason for these significant differences in the lateral and deep coagulation is that Wilder-Smith study used a much smaller diameter tip, producing a spot size 300 microns versus our tip diameter 0.08 mm, a size commonly used in a clinical setting. Other possible reasons are unknown microscope magnification used to perform measurements in the Wilder-Smith study, and the 30 mm in length incisions were timed with a stopwatch for 4 seconds, delivering less laser energy than in a clinical setting utilized by our study. Our time to make a 22 mm long incision ranged from 4.5 to 5.9 seconds.

The depth of incision showed scalpel making the largest cut, because the incision was made down to the bone. The carbon dioxide and Er,Cr:YSGG lasers resulted in less incision depth, as expected due to high water absorption in soft tissue. The diode laser was inconsistent and had a wide range of the depth of incision. The Nd:YAG laser had the most incision depth.

The width of incision showed the Nd:YAG laser making the most. The width of incision for the carbon dioxide laser was not statistically significant from the Er,Cr:YSGG laser, and the diode laser. Scalpel made the least incision in the soft tissue.

The depth of coagulation is non-existent in the scalpel incision. Both the carbon dioxide and the Er,Cr:YSGG lasers are significantly different, having less depth of coagulation than the diode and the Nd:YAG lasers. Also the diode laser is significantly different from the Nd:YAG laser, with the diode exhibiting less depth of coagulation.

The lateral coagulation in the scalpel incision was zero. Er,Cr:YSGG laser was significantly different with less lateral coagulation, only from the diode laser (which had much more variability). In this study, the carbon dioxide laser had a very wide mean lateral coagulation.

Lateral and deep coagulation includes: the zone of coagulation, the zone of stasis, and the zone of edema, because these zones are not distinguishable during microscopic examination under 10x magnification used in this study. Thus, the irreversible tissue changes of the coagulation zone are included with the reversible tissue changes. The width of incision and the depth of incision linearly defines the three dimensional ablation defect, appearing as irreversible tissue loss. Our findings for the four lasers and the scalpel used to make a soft tissue incision are similar to the findings in other studies from the 1990s and the 2000s (4, 6, 7, 8, 9, 11).

We used four different types of lasers according to each manufacturers specifications for the soft tissue incision, with varied tip diameter, ranging from 320 microns tip diameter for the Nd:YAG laser to 0.8 mm diameter tip for the carbon dioxide laser. The data showed that the wavelength, and its consequent interaction with the tissue (non-water absorbent or water absorbent) determines the linear size of the zones of laser-tissue interaction.

The statistical significance confirms the clinical findings that the choice of a scalpel or one of the four lasers should be based on the desired clinical effect during soft tissue incision. When the primary closure of the incision is called for, the stainless steel scalpel blade will accomplish the incision with minimal soft tissue loss, or carbonization at the wound edges, such as in soft tissue grafting and incisions in thin gingival. When the hemostasis is highly desired clinically, such as in gingivectomy, frenectomy, or any periodontal disease treatment procedures then any of the four laser types can be used, keeping in mind the wavelength of each laser and its water absorption. Deeply penetrating into the soft tissue, and with low water absorption, are the diode and Nd:YAG lasers. High water absorption, and less deep penetration into the soft tissue characterizes the carbon dioxide and Er,Cr:YSGG laser. When the primary closure of the incision is not desired by the clinician, a laser provides hemostasis, an incision is suture free, and the laser aided procedure is better accepted by the patient.

The stainless steel scalpel blade incision was made in the least amount of time. It takes time for the laser light and soft tissue to interact, and it is clinically important to assess the size of soft tissue incision one wishes to make. Any type of the four lasers tested in our study has been used as an adjunct to the scalpel incision for the hemostasis and the coagulation effect, such as in the periodontal disease treatment.

CONCLUSION

Our study supports the clinical experience that the choice of scalpel or one of the four lasers tested for the soft tissue incision depends on the goal of the surgical effect desired. Lasers are used for hemostasis due to their coagulation effects, with the diode and Nd:YAG lasers exhibiting the most lateral and deep coagulation in the soft tissue due to their low water absorption. The carbon dioxide and the Er,Cr:YSGG lasers are highly absorbed in water, and their lateral and deep coagulation is lesser. However, all laser types induce similar effects clinically in the soft tissue incision as compared to the stainless steel scalpel blade.

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