INSTITUTO DE PESQUISAS ENERGÉTICAS E NUCLEARES
Autarquia Associada à Universidade de São Paulo

Thermal damage and time of excision of micro and super pulsed diode lasers: an *ex-vivo* comparative study

MARILIZA CASANOVA DE OLIVEIRA PRADO

Tese apresentada como parte dos requisitos para obtenção do Grau de Doutor em Ciências na Área de Tecnologia Nuclear - Materiais

Orientadora:
Profa. Dra. Denise Maria Zezell

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“The real voyage of discovery consists not in seeking new landscapes, but in having new eyes.”.

(Marcel Proust)

RESUMO

OLIVEIRA-PRADO, Mariliza C. ***Dano térmico e tempo de excisão dos lasers de diodo micro e superpulsado:*** um estudo comparativo *ex-vivo*. 2021. 138 p. Tese (Doutorado em Tecnologia Nuclear) - Instituto de Pesquisas Energéticas e Nucleares, IPEN/CNEN-SP, São Paulo.

Os *lasers* de diodo são unidades cirúrgicas portáteis, acessíveis, eficientes e confiáveis na realização de cirurgias e biópsias de tecidos moles da cavidade oral. As unidades mais atuais estão equipadas com tecnologias micro e superpulsadas e sistemas que permitem a seleção de diferentes parâmetros de irradiação quanto ao modo de emissão, ciclo de trabalho, potência e duração de pulso. Desconhece-se, no entanto, quais parâmetros ou tipos de *lasers* de diodo produzem as amostras teciduais mais íntegras no melhor tempo cirúrgico possível. O objetivo principal deste estudo *ex-vivo* foi comparar o dano térmico e o tempo de excisão de diferentes parâmetros do *laser* de diodo micropulsado e do *laser* de diodo superpulsado. Os objetivos secundários foram: 1) prover recomendações práticas para realização de excisões cirúrgicas com *lasers* de diodo; 2) examinar possíveis correlações entre a área e a profundidade do dano térmico, bem como entre o tempo de excisão e o dano térmico. Dez grupos de dez espécimes de línguas suínas foram excisionados (8 mm de diâmetro) usando lâmina cirúrgica (grupo controle: G1); *laser* de diodo micropulsado em diferentes modos de emissão, ciclos de trabalho, potências médias, potências pico e durações de pulso (G2 - 9); e o novo *laser* de diodo superpulsado (G10) no menor parâmetro único recomendado pelo fabricante (potência média = 3,2 W, potência pico = 80 W, duração de pulso de 10 µs a 100 ms). O comprimento de onda de ambos foi de 940 nm. Todos os parâmetros foram previamente medidos com o medidor de potência power meter, e todas as pontas descartáveis previamente ativadas foram descartadas logo após a realização de cada biópsia. A área e a profundidade histológica do dano térmico foram quantificadas através do software *NIS-Element Basic Research* (Nikon Instruments Inc), enquanto que o tempo de excisão foi medido entre o pinçamento até a excisão total da lesão. Os testes não paramétricos de Kruskal-Wallis e de comparação múltipla de Dunn com correção de Bonferroni foram aplicados para comparar a área e profundidade de dano térmico, assim como o tempo de excisão entre os grupos. As correlações entre área e profundidade do dano térmico e entre o tempo de excisão e o dano térmico foram examinadas através do coeficiente

de correlação não paramétrico de Spearman. O nível de significância estabelecido foi de 5%. Nos grupos experimentais (G2 - G10), a área total de dano térmico observada foi menor no grupo G3 (modo contínuo, potência média = 1,5 W; mediana = 0,91 mm^2; p = 0,009). Todos os outros grupos apresentaram áreas de dano térmico superiores a 1 mm^2 com G7 (modo pulsado, ciclo de trabalho = 33%, potência média = 1,5 W, pico = 5,4 W e duração de pulso de 100 µs) e G9 (modo pulsado, ciclo de trabalho = 50%, potência média = 1,5 W, potência pico = 3,6 W e duração de pulso = 1 ms) produzindo as maiores áreas de dano (mediana de 1,93 e 1,97 mm^2, respectivamente). Na comparação múltipla, controlando o nível de significância global, G3 apresentou área mediana de dano térmico significativamente menor que G7 (p = 0,013) e G9 (p = 0,036). Não foram observadas diferenças estatisticamente significativas na profundidade do dano térmico entre os grupos (p = 0,12). Os tempos medianos de excisão de G1 (bisturi) e G10 (superpulsado) foram significativamente menores do que os encontrados nos grupos de *laser* de diodo micropulsado (G1 = 50; G10 = 69; G2 a G9 variaram de 142 a 238 segundos; $p < 0,001$). Houve correlação direta entre a profundidade e área de dano térmico, porém não foi encontrada correlação entre o tempo de excisão e o dano térmico. O uso do *laser* de diodo micropulsado em modo contínuo com potência média = 1,5 W produziu biópsias com a menor área de dano térmico e maior integridade tecidual, enquanto que o uso do *laser* de diodo superpulsado (potência média = 3,2 W, potência pico = 80 W, duração de pulso de 10 µs a 100 ms) permitiu excisões mais rápidas. Área e profundidade de dano térmico correlacionaram-se diretamente. Na prática clínica, recomenda-se o *laser* de diodo micropulsado no modo contínuo, na potência média de 1,5 W como a melhor opção quando se objetiva atingir integridade tecidual máxima. Por outro lado, o uso do *laser* de diodo superpulsado com potência média = 3,2 W, potência pico = 80 W, duração de pulso de 10 µs a 100 ms, produz a melhor relação entre dano térmico e tempo de excisão, devendo ser utilizado quando a necessidade prática requerer excisões rápidas com integridade tecidual razoável. Deve-se evitar o uso do *laser* de diodo micropulsado em modo de emissão pulsado com altas potências pico e longas durações de pulso na realização de biópsias orais a fim de que a integridade tecidual não seja comprometida e dificulte a análise histopatológica das lesões.

Palavras-chave: *lasers* de diodo; tecido mole; dano térmico; tempo de excisão, biópsia oral; *laser* de diodo micropulsado; *laser* de diodo superpulsado.

ABSTRACT

OLIVEIRA-PRADO, Mariliza C. **Thermal damage and time of excision of micro and super pulsed diode lasers**: an *ex-vivo* comparative study. 2021. 138 p. Thesis (Ph.D. in Nuclear Technology), Nuclear and Energy Research Institute, IPEN-CNEN/SP, São Paulo.

Diode lasers are portable, accessible, efficient and reliable surgical units for performing surgeries and soft tissue biopsies of the oral cavity. The newer units are equipped with micro and super pulsed technologies and systems that allow the selection of different usage parameters in terms of emission mode, duty cycle, power and pulse duration. It is, however, not known which parameters or types of diode lasers produce the most intact tissue samples in the best possible surgical time. The main objective of this *ex vivo* study was to compare the thermal damage and the excision time of different parameters of the micro pulsed diode laser and the super pulsed diode laser. The secondary objectives were: 1) to provide practical recommendations for performing surgical excisions with diode lasers; 2) to examine possible correlations between the area and the depth of the thermal damage, as well as between the time of excision and the thermal damage. Ten groups of ten swine tongue specimens were excised (8 mm in diameter) using a surgical blade (control group: G1); micro pulsed diode laser in different emission modes, duty cycles, average power, peak power and pulse duration (G2 - 9); and the new super pulsed diode laser (G10) with the smallest single parameter recommended by the manufacturer (average power = 3.2W, peak power = 80 W, pulse duration from 10 μs to 100 ms). The wavelength of both was 940 nm. All parameters were previously measured with the power meter and activated disposable tips were discarded right after each biopsy. The area and the histological depth of the thermal damage were quantified using the NIS-Element Basic Research software (Nikon Instruments Inc), while the excision time was measured between the clamping until the total excision of the lesion. Kruskal-Wallis and Dunn's multiple comparison tests with Bonferroni correction were applied to compare the area and depth of thermal damage, as well as the time of excision between the groups. Correlations between area and depth of thermal damage and between excision time and thermal damage were examined using Spearman's nonparametric correlation coefficient. The level of significance was set at 5%. In the experimental groups (G2 - G10), the total area of thermal damage observed was smaller in the G3 group (continuous mode, average

power = 1.5W; median = 0.91 mm^2; p = 0.009). All other groups had thermal damage areas larger than 1 mm^2 with G7 (pulsed mode, duty cycle = 33%, average power = 1.5W, peak = 5.4W and pulse duration of 100 µs) and G9 (pulsed mode, duty cycle = 50%, average power = 1.5W, peak = 3.6W and pulse duration = 1 ms) producing the largest damage areas (median 1.93 and 1.97 mm^2, respectively). In the multiple comparison, controlling the level of global significance, G3 presented a median area of thermal damage significantly smaller than those of G7 (p = 0.013) and G9 (p = 0.036). There were no statistically significant differences in the depth of thermal damage between the groups (p = 0.12). The median excision times of G1 (scalpel) and G10 (super pulsed) were significantly shorter than those found in the micro pulsed diode laser groups (G1 = 50; G10 = 69; G2 to G9 ranged from 142 to 238 seconds; p < 0.001). There was a direct correlation between the depth and area of thermal damage, but no correlation was found between the excision time and thermal damage. The use of the micro pulsed diode laser in continuous mode with average power = 1.5 W produced biopsies with the smallest area of thermal damage and greater tissue integrity, while the use of the super pulsed diode laser (average power = 3.2W, peak power = 80W, pulse duration from 10 µs to 100 ms) allowed faster excisions. Area and depth of thermal damage correlated directly. In clinical practice, the micro pulsed diode laser should be considered in continuous mode, at 1.5 W output power with 1.8 W peak power as the best option when aiming to achieve maximum tissue integrity. Whereas the use of super pulsed diode laser with output power = 3.2W, peak power = 80W, pulse duration from 10 µs to 100 ms, produced the best relationship between thermal damage and excision time, and is recommended when practical necessity requires rapid excisions with reasonable tissue integrity. The use of micro pulsed diode laser in pulsed emission mode with high peak power and long pulse duration should be avoided when performing oral biopsies so that tissue integrity is not compromised and hinders the histopathological analysis of the lesions.

Keywords: diode lasers; soft tissue; thermal damage; excision time, oral biopsy; micro pulsed diode laser; super pulsed diode laser.

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LIST OF SYMBOLS

λ	Wavelength
°C	Degrees Celsius
Hz	Hertz
IV	Infrared
μm	Micrometers
%	Percentage
P	Power
UV	Ultraviolet
W	Watts
μ_a	Absorption Coefficient
μ_s	Scattering Coefficient
CW	Continuous Wave
CP	Continuous Pulsed
STP	Super Thermal Pulsed

SUMMARY

1 INTRODUCTION

The main scope and basis for the development of this study was the use of diode laser as an advanced surgical method for the removal of benign lesions in the oral mucosa. For this reason, the initial concepts of this study were guided by laser in the field of dentistry.

Laser is a device that stimulates atoms or molecules to emit light at particular wavelengths. This light consists of a very narrow and concentrated beam of radiation which, depending on the type of laser and target tissue to which it is directed, may result in thermal effects.[1]

The principles that led to the development of *LASER* (*Light Amplification by Stimulated Emission of Radiation*) were initially described by Albert Einstein in 1917, with the publication of his treatise “Zur Quantum Theorie der Stralung”.[2] However, it was only in 1960 that Theodore Maiman, an English physicist, developed the first equipment using a ruby crystal with *MASER* (*Microwave Amplification by Stimulation Emission of Radiation*) partially based on the previous researches of Charles Townes.[3-6] Both *Laser* and *Maser* are systems that are based on the amplification of light by the interaction between photons (quantum of the electromagnetic field; that is, massless light particles that propagate forming an electromagnetic wave).[3] In 1964, Stern and Sognnaes conducted the first experiments in the field of dentistry when they made use of ruby laser in dental tissues “*in vitro*”, in which they observed changes in temperature in the irradiated tissues.[7] Later, in 1966, Goldman *et al* used the same laser clinically, on enamel and dentin.[8, 9]

At present, laser occupies an indispensable place as an instrument vastly used science and technology, attaining its clinical application in the fields of medicine and dentistry. Differently from other light sources, lasers have unique characteristics, because they emit electromagnetic radiation in the form of collimated flow of light (parallel light beams), coherent (synchronism of photons in time and space), and monochromatic (a single wavelength).[8] These characteristics enable various applications of the device in the most diverse areas of health [care].[10-13] In dentistry,

laser is widely used in the identification, differential diagnosis and treatment of oral lesions.

Lasers can also be classified according to their wavelength and light emission characteristics into low power and high power systems. Low power (level) lasers are frequently used for wound repair treatments, decontamination of root canals with exogenous pigments (methylene blue), among others. Whereas high power (level) lasers have been used in stomatology for the treatment of angiomatosis lesions, since the decade of the 1960s.[14, 15] Among these, diode laser, in different wavelengths (810-1064 nm), has been useful for performing innumerable procedures that involve both biopsies for histopathological diagnosis of oral lesions[14, 16, 17] and treatment of mucocele, neoplastic lesions, Fordyce granules, fibromas, hyperkeratoses, in addition to gingivectomies, frenectomies and gingivoplasty procedures.[18-22]

Over the last few decades, with the growing clinical experience with the use of laser technology in oral surgery, this technique has been demonstrated to be more advantageous that use of the conventional scalpel. This is because it allows procedures to be performed in minimally invasive mode, which has generated multiple benefits for both dental surgeons and patients,[18, 20, 23-25] thereby transforming laser into a valuable instrument for performing soft tissue surgeries, including biopsies.[10, 11, 13] Among its benefits are decontamination of the operative field, control of bleeding in both trans- and post-operative periods, improved visualization of the surgical field, reduction in performing sutures and use of local anesthetics, in addition to significant reduction in both the inflammatory process and pain in the post-operative period.[10-13, 16, 26-29]

The main difference between a laser cut and the type performed with a scalpel is the formation of a layer of coagulated tissue in the depth of tissue walls submitted to laser incision.[30-32] The unique interaction of diode lasers with the pigments of oral soft tissues has allowed that they configure as a most selective surgical tool that produces controlled and minimally invasive surgical results. After all, this determines the innumerable benefits previously mentioned (improvement in hemostasis, reduction of inflammation and pain, improvement in the cicatricial process, among others)[14, 33] Because of these specific characteristics, diode lasers surpass the majority of

conventional techniques such as use of the scalpel and electrocautery processes.[16, 26, 33-36]

When diode lasers are used without an adequate protocol, they may produce thermal effects on interacting with the target tissues. Similar to other high intensity lasers, diode lasers convert light into thermal energy, producing heat in the tissue, causing a zone of thermal damage around the incision, which may attain the stage of carbonization.[28, 37] This laser-tissue interaction determines different biological effects, such as hyperthermia, coagulation, vaporization and carbonization.[38, 39] This effect, denominated the photothermal effect, produces changes in the tissue on being examined by optical microscope. The damage caused by laser light energy may make it difficult to achieve histopathological interpretation. Therefore, precise control of the temperature generated by laser is essential.[25, 30]

High intensity laser can emit light in two ways: continuous and pulsed emission. Diode Lasers, in particular, have a constant emission of radiation, and may induce tissue damage by excessive heat in the target tissue, and interfere negatively in the histopathological assessment and final diagnosis of specimens evaluated.[37, 38] In some of the older diode devices, a mechanical obturator blocks the transmission of light, producing pulses that allow thermal relaxation of the target tissue.[39, 40] This resource causes the energy to be delivered in repetitive pulses that vary, in general, from tens of milliseconds (ms) to hundreds of microseconds (µs) or other methods that produce even shorter pulses. Thus, lasers with shorter duration of pulses determine lower tissue temperatures and, therefore, may produce less carbonization of the target tissue.[41]

The determinants of the effects of laser on biological tissues are the wavelengths, peak power and pulse width.[42, 43] High power (level) diode lasers are portable surgical units with an accessible cost, providing benefits in soft tissue surgeries. The more modern and up-to-date diode devices are equipped with super and micro pulsed technologies that control the duty cycle (fraction of time expressed in percentage in which the laser is actively emitting energy).[44, 45] This allows the laser to deliver high power in a short period of time, protecting the tissue from potential damage caused by heat. Furthermore, to minimize the thermal damage to adjacent structures, it is necessary to adjust the pulse duration of laser and its peak power,

which prevents increase in the tissue thermal effect and reduces the risk of carbonization. According to some authors, the degree of thermal damage depends on the time of thermal relaxation of tissue produced by the laser.[46, 47]

At present, the diode lasers most frequently used in dental clinical practice are equipped with systems that allow combined selection of different irradiation parameters (emission modes, duty cycles, output power, peak power and pulse duration) in order to enable the light beam to be modulated to meet the practical needs of dental surgeons in performing procedures such as oral biopsies.[48, 49] However, selection of the parameters for obtaining whole samples in adequate surgical times is performed in a subjective manner, as there are no practical recommendations based on consistent studies that have comparatively analyzed different types and combinations of diode laser parameters. The few studies conducted up to now have shown considerable limitations, such as small sample sizes and reduced number of parameters assessed.[50-53] Therefore, an in depth comparative analysis of thermal damage and time of excision produced by different types and combinations of diode lasers is necessary.

2 OBJECTIVES

2.1 General Objective

The main objective of this *ex-vivo* study was to compare the thermal damage and time of excision of different micro pulsed diode laser and super pulsed diode laser parameters.

2.2 Specific Objectives

- Establish micro pulsed and super pulsed laser parameters that produce less thermal damage through descriptive (area of tissue thermal change) and morphometric (area and depth) analysis of the excised tissue.
- Identify the type of laser and the parameter that determines the shortest time of excision, as well as the conventional scalpel.
- Identify whether there is correlation between the time of excision by diode laser and the thermal damage produced.
- Identify whether there is correlation between the area (mm^2) and depth (µm) of thermal damage.

3 ASPECTS OF ORIGINALITY

The originality of this study is based on the absence of practical recommendations for selecting micro pulsed and super pulsed diode laser parameters that enable whole excisional biopsies to be obtained by laser in an appropriate amount of surgical time spent. Therefore, the present study makes it possible for dental surgeons, who make use of diode lasers. to select the best types and combinations of lasers in an objective manner, well founded on comparative data, so that the results obtained can have positive implications on future clinical practice.

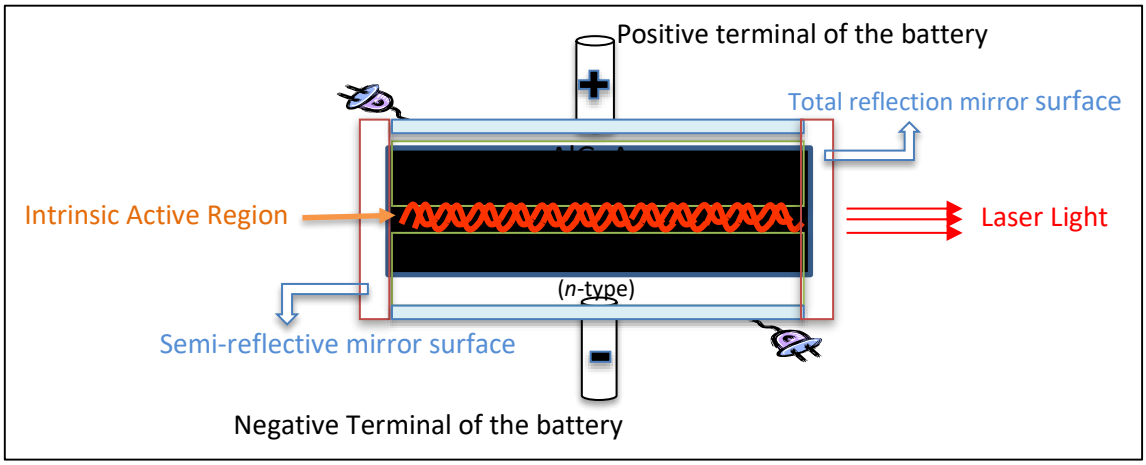
4 REVIEW OF THE LITERATURE

4.1 Diode Laser

Diode lasers convert electric current directly into light. They are solid-state semiconductor devices that function with a system of layers of different semiconductor materials. Their system has a layer of positive electrical charge conducting material (layer *p*) that is separated by another layer of negative charge conducting material (layer *n*) considered a non conductive layer (Figure 1). Application of negative voltage current to layer *n* and concomitant application of positive voltage current to layer *p*, causes the phenomenon of exchange occurs in the non conductive layer, allowing the recombination of the carriers (electrons and electron-hole), resulting in release of energy in the form of light. Therefore, recombination of the pair electron-hole corresponds to a transition of electron between the valence band and conduction band, leaving a “hole” than can carry both a current and a charged physical particle. This being so, in recombination an electron of the conduction band closes the energy and reoccupies the state of energy of a electron hole in the valence band. It is necessary to activate surfaces coated with mirrored material to capture the light photon for the purpose of creating an optic cavity and thus allow the diode laser to be a former of laser radiation.[3] This technology has allowed the development of compact, portable diode lasers that are easy to operate, and low cost in comparison with laser equipment used in hard tissues, based on active gaseous or solid media.[16, 54-56]

The active medium in solid state of the semiconductor diode laser is the portion that defines the wavelength. When these active media receive the energy in the form of difference of potential (threshold current) they release it in the form of photons.[3] As is the case with the other lasers, diode laser produces collimated, coherent and monochromatic radiation which, when absorbed by the target tissue, increases its temperature, and produces photothermal effects.[57, 58]

Figure 1 – Diagram of semi-conductor laser structure in the production of laser light



Source: The author.

Diode laser was introduced into Dentistry and oral surgery in the decade of the 1990s.[59] At present, there are various wavelengths for diode laser, but those most frequently used in Dentistry are the Aluminum Gallium Arsenide (AlGaAs) (810 nm), Gallium Arsenide (GaAs) (940 nm), and the Indium Gallium Arsenide (InGaAs) (980 nm), which operate in the invisible band close to the infrared wavelength. The quantity of energy that is absorbed depends on the characteristics of the tissue, such as pigmentation, water content, and on the laser wavelength and its emission mode. Tissue compounds called chromophores preferentially absorb certain wavelengths.[60]

The choice of diode laser consists of good light absorption by tissues rich in chromophores, generating good results relative to hemostatic control.[12, 61] Therefore, wavelengths related to non-visible diode lasers and those close to infrared are adequately absorbed by pigmented tissue that contains melanin and hemoglobin. However, are hardly absorbed by calcified tissues such as hydroxyapatite.[62] The interaction of diode laser in tissue makes it considerably safe and well indicated for oral soft tissue surgeries in regions close to the dental structures.[63]

In the majority of general dental practices, diode lasers are emerging as coadjuvant to the traditional tools of simple soft tissue surgeries, such as excisional biopsies.[3, 35, 36, 64] Surgical predictability, absence of bleeding, shorter period of surgical time, lower quantity of anesthetics, post-operative period without pain, precision of the procedure, greater comfort for the patient, and reduction in the use of

sutures are stimuli to professionals for incorporating this new modality of oral treatment into their practices.[26, 27, 33-35] The hemostatic property of laser is extremely important in the excision of an exophytic lesion; It may be used to interrupt bleeding in the field due to its capacity for contracting the collagen of the vascular wall.[65, 66]

Diode laser is an adequate therapeutic device for excision of oral lesions larger than 3 mm in diameter, but may cause severe thermal effects in small lesions.[64] However, other studies have suggested that the samples must measure at least 5 mm in diameter in order to obtain a reliable evaluation of the histological sample.[67]

Laser light beams may be emitted in continuous or pulsed mode.[13, 30, 68] The emission modes describe the manner how the laser energy travels through time. The main benefit of pulsed mode is the capacity of the target tissue to cool between the successive pulses. In this mode the duration of the pulse occurs from 0.1s to 0.001s. Generally, the continuous mode is the fastest manner of eliminating tissue because the laser energy is emitted continuously without any time interval, generating heat which could accumulate and cause damaging side effects in both the target and adjacent tissues. It may also be used, according to the clinical indication, in contact mode or without coming into contact with the tissue.[61, 69]

The wavelengths of 600-1000 nm presented in low power (level) lasers (therapeutic treatments) and high power (level) (surgical treatments) are commonly used in pulsed or micro pulsed mode.[17, 18, 56]

These wavelengths are safe, and indicated for therapeutic and surgical procedures in soft tissues in regions close to dental structures such as incision, vaporization, curettage, blood coagulation and hemostasis.[39, 54, 62, 70-72]

When correct irradiation parameters are used, a central zone of tissue ablation is surrounded b an area of irreversible protein denaturation (coagulation). Around this central zone, a reversible and reactionary area of edema will develop along a thermal gradient. Ideally, the line of incision will be equal to the beam diameter.[17]

Heat accumulation plays a role in decontamination of the target and surrounding area, and in the production of a surface clot, inhibiting bacterial contamination in the wound. When using lasers, which emit in the near infrared range, in soft tissues, there is minimal or no bleeding due to a combination of sealing small vessels by means of

protein denaturation in the tissue, and stimulation of Factor VII production in the clot. The increase in heat also allows sealing of small lymphatic vessels that results in a reduction in post-operative edema. Suturing is generally also unnecessary due to the superficial clot.[17, 30]

4.2 Interaction of high power laser with soft tissues of the oral cavity

The interaction of a laser beam with a biological tissue depends on factors related to the properties of laser radiation, and thermal and optical properties of the tissues.[3] Wavelength, emission mode (continuous or pulsed), peak power, average power, repetition rate, focused area of the laser beam (energy density and power), pulse duration, quantity of energy delivered, delivery mode (fiber optic, articulated arm), mode of application (with or without contact, focused or not, in circular movements or without displacement) presence or absence of cooling system and time of exposure, when they interact with the biological tissue are considered the parameters and properties of the laser beam. Furthermore, the following must be considered optical properties of the tissue: the coefficients of absorption, reflection and scattering; and as thermal properties, there are thermal conductivity and thermal capacity of the tissue.[3, 42, 50]

Composition of the tissue and thermal relaxation are also attributes to influence the laser-tissue interaction.[14, 43, 73]

Electromagnetic radiation may act on biological tissue in various ways, and one of these is incidence of the light beam in a proportion of the material. There are five effects that may occur as a consequence of the interaction of electromagnetic radiation with biological tissues: reflection, refraction, absorption, scattering and transmission.[62, 74, 75]

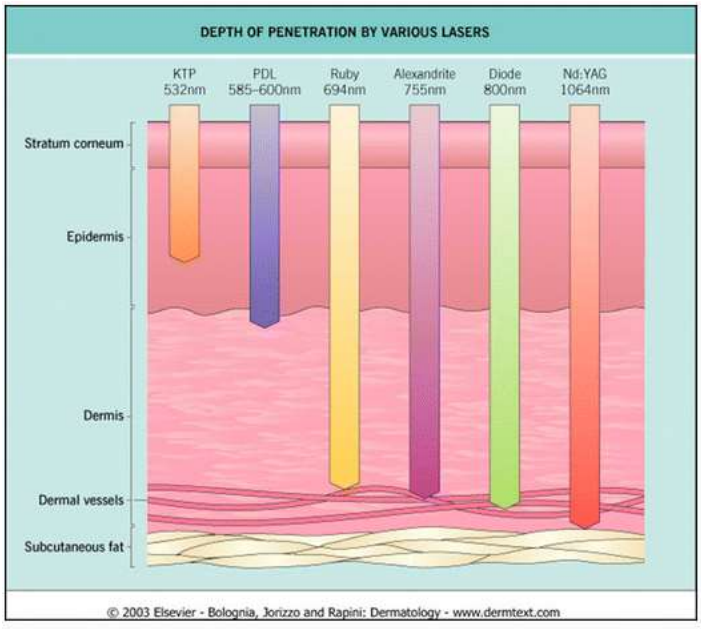
In the process of the interaction between laser light and biological tissue, it is important to consider that its effectiveness will only occur when the photons arising from the laser equipment were absorbed by the electrons of the atoms in the target tissue.

When the incident energy of the laser beam is attenuated by the medium and transferred to it, this is known as absorption; that is, the electromagnetic wave will not return to the incident surface and will not propagate itself in the medium. Thus we have the situation that the laser wavelength is resonant with the irradiated biological tissue.[75, 76]

The wavelength determines both the absorption by a certain chromophore and the depth of penetration of the light. Therefore, the laser wavelength, for example, in the non visible infrared band, as is found in high power diode lasers for clinical use, covers the range from 810 to 1064 nm. The greater the wavelength, the greater the optical penetration of the laser light into the tissues, in this range since the absorption of the chromophores diminishes.[77] (Figure 2).

The deepest penetrations are found in the wavelengths from 800 nm to 1.100 nm. From this region, the Mid- and Far- infrared radiation is absorbed almost entirely by the water of the epidermis (which is made up of 90% water) and in this case there is less penetration. Penetration into the tissues also depends on the diameter of the laser beam in the region of focus (spot size) and varies with the laser wavelength.[77]

Figure 2 – Representation of comparative penetration depth of various lasers into biological tissues



Source: Bolognia et al. [78].

When the incidence of laser light occurs on tissues, it either penetrates or is reflected, as shown in Figure 3.[3] When laser incides on soft tissue of the oral cavity, its irregularities (roughness) are assumed to be large, when compared with the wavelength of incident radiation, and thus, the type we call diffused reflection occurs. Therefore, reflection is defined as electromagnetic radiation that incides on a surface and returns to the medium of origin because of its density, or the because angle of incidence is smaller than the angle of refraction. The reflective surface is a physical interface between two materials with different refraction indexes such as, for example, air and tissue.[3, 75]

When the laser light beam incides on a medium and is transmitted because no interaction occurred between the two of them (incident beam and medium), this is denominated refraction. The tissue only transmits the photons that are not reflected, absorbed or scattered, in the same direction as that of the incident beam. This phenomenon, which we know as transmittance, was derived in this way, and it is defined as being the ratio between the intensities of radiation transmitted and incident radiation. Both the phenomenon of reflection and refraction are strongly inter-related by the Law of Fresnel.[75]

The effect of transmission refers to the ability of energy to be diffused by means of the tissue without any effect or these photons are scattered, in which case they change direction, and are capable of returning to the same direction as that of the beam entrance. This ability of energy to diffuse in the medium without any effect is related to the type of tissue and wavelength.[79]

The motion of charged and elastically confined particles exposed to the electromagnetic waves, occurs according to the incident electric field. The wave frequency that does not correspond to the natural frequency of the particles, gives rise to the process of scattering. In this process, a reduction in energy occurs with the distance of propagation, together with a distortion of the beam, in which the photons proceed in a disorderly direction through the medium.[74, 75]

In the process of laser light penetration into the tissue, the most important effect is that of absorption in order to enable the useful therapeutic effect to occur.

When incidence of a laser beam on biological tissue occurs, the part that penetrates undergoes attenuation by absorption and scattering, as it travels into the material. The effects of absorption and scattering are characterized by the coefficients of absorption (μ_a) and scattering (μ_s) that represent the rate of loss of radiation energy per unit of penetration depth, due to absorption and scattering of photons, respectively. Both coefficients are specific for each type of tissue and each wavelength.[74]

In order to describe the effect of thickness and of concentration on the phenomenon of absorption, the Law of Lambert-Beer is used. (Equation 1). In which *z:* is the optical axis; *I(z):* represents the intensity in the distance z; $I_{0:}$ represents the incident intensity; *α:* coefficient of absorption in the medium.[62, 75]

$$I(z) = I_0 \, e^{-\alpha z} \qquad (1)$$

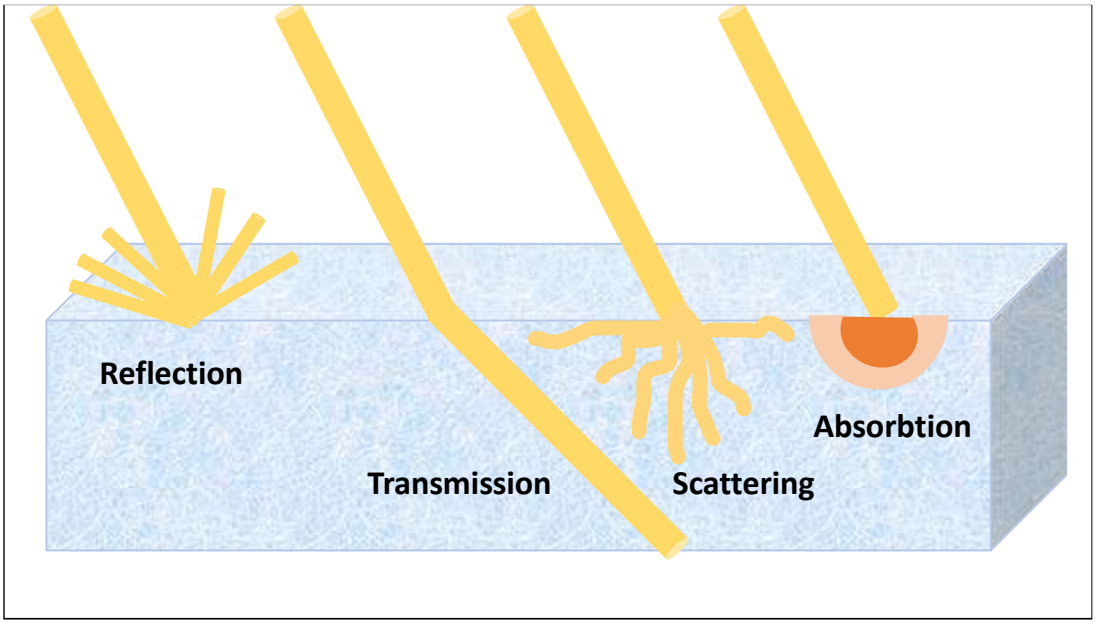
The length of absorption of the target tissue was defined as being inverse to the coefficient of absorption (μa), and corresponds to the depth of penetration to which 63% of the incident light was absorbed by the target tissue. Therefore, the remaining light beam has 37% of the intensity of the initial beam.

In biological tissues, absorption depends on composition of the tissue, pigmentation and its water content. The components of tissue that have a high coefficient of absorption at a specific wavelength, or in a certain region of the electromagnetic spectrum, are denominated chromophores (Figure 4). A most important factor for good surgical effect in tissue is the absorption of laser light by the target tissue. The chromophores perform a fundamental function in the interaction of radiation with the tissue.[79]

The composition of tissue, its water content, pigmentation and wavelength are essential components for enabling the effect of absorption to occur. The effects of the laser beam occur by means of this energy being transferred from the incident photons to the tissue during the process of absorption.[3]

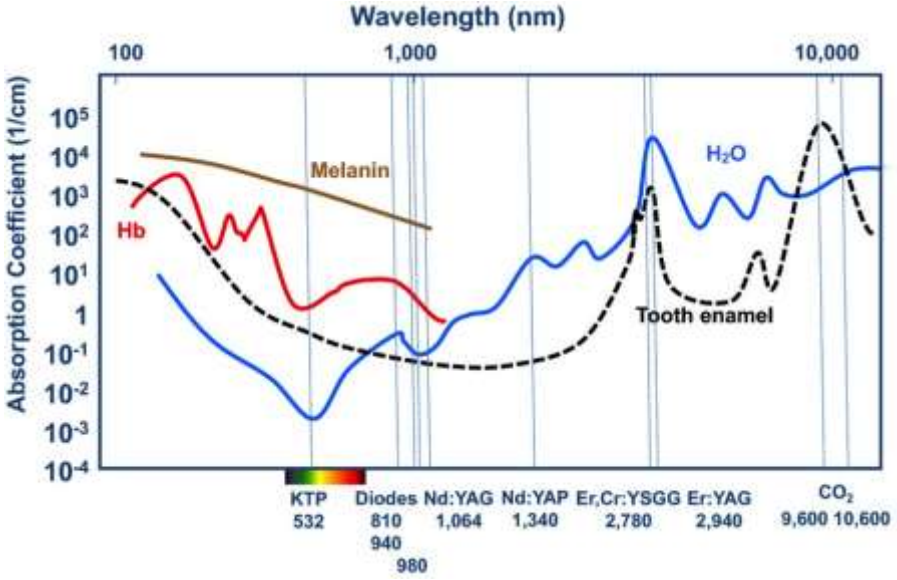
Among the mechanisms of interaction, when laser light incides on biological tissue, the energy absorbed is transformed into other non optical forms of energy, capable of producing four types of effects: photothermal, photochemical, mechanical and electrical. The laser parameters are composed of this multiplicity of interactions, and the specific characteristics of each type of tissue.[14]

Figure 3 - Interaction of laser light incident on biological tissue – possibility of events



Source: Adapted from Sener [80],

Figure 4 - Approximate absorption curves of main components of biological tissues relative to wavelength. In the vertical lines, the following lasers are highlighted: KTP lasers (Potassium titanyl phosphate), Diode, Nd:YAG (Yttrium aluminum garnet doped with neodymium) Nd:YAP (Neodymium: Yttrium-Aluminum-Perovskite), Er,Cr:YSGG (Chromium Erbium doped Yttrium Scandium Gallium Garnet), Er:YAG (erbium doped Yttrium aluminum garnet) and CO_2 (carbon dioxide), with affinity for water (H_2O), tooth enamel, melanin and hemoglobin (Hb)



Source: Coluzzi [81, p.9].

There may be different types of photochemical effects such as photodynamic, photoinduction, photoactivation and photobiomodulation therapy. All of these processes are the result of low absorption of energy by the tissue, making it possible to activate biochemical processes within the cell.[3]

When the absorption of laser light by the tissue, and its transformation into thermal energy occur, we have what is known as the photothermal effect. Laser light is absorbed and transformed into heat.[62] This heat will induce an increase in molecular vibration, enlarging the inter-atomic space and total energy of the tissue, causing the phenomenon of thermal expansion.[82]

The reason why the relations between penetration depth and energy density must be taken into consideration in order to understand the relations between laser power and tissue temperature, is the production of various gradients of temperature during production of the effect of laser absorption by the biological tissue.[79] However, the high temperatures caused by absorption of laser energy is diffused through the

tissue according to its thermal properties after being transformed into heat.[3] The loss of heat to unexposed structures of the tissue is denominated thermal conductivity.[62]

Thermal conductivity depends on the quantity of water and blood flow in the tissue; Authors have affirmed that blood flow acts as a “reservoir” of heat that drains the thermal energy to other locations.[79]

When considering the area in which heat is propagated and the increase in temperature generated after the absorption of photons from laser, some effects may occur such as photothermolysis, photohyperemia, photocoagulation and photoevaporation. All of these effects are of a thermal nature, and may occur in layers or in areas that were not directly attained by laser light, depending on the depth of penetration.[3]

The lasers that give rise to photothermal effects are guided by the principles of clinical application, first described by Anderson and Parrish[83] in which selective photothermolysis produces the thermodynamic effects observed when heating occurs in a reduced space and includes: 1. The laser light wavelength chosen must be selective and appropriate for use in the target tissue; 2. The target tissue must be treated without damaging the surrounding tissues; that is the diode laser pulse width must be within or below the time of thermal relaxation of the tissues treated. Therefore, it is important to adjust the laser pulse width, in order to minimize the thermal damage to adjacent structures.

4.3 Influence of laser pulse duration on biological tissues

High power lasers used for irradiating oral tissues have aroused great interest in the Dentistry Community and Scientific World. Parameters used such as: wavelength, power, power density, time of exposure, pulse duration, emission mode are inter-related to the effects that this irradiation with laser could cause in the target tissue.[40, 84]

The possibility of irreversible thermal damage to the oral tissues is prevented when adequate and reliable parameters are used for irradiating biological tissues.[85]

The width and intensity of thermal damage may be subject to the characteristics and parameters of the laser (average power, wavelength, type of optic fiber used and affinity for target tissue). Whereas the power, frequency, pulse duration and fiber diameter may be involved in the efficiency of the cut.[18, 61, 86]

The pulse duration refers to the time in which the biological tissue is exposed to irradiation with laser.[87] Cooling of the surface is known as the time of thermal relaxation. According to Hayes and Wolbarsht (1968)[46] e Wolbarsht (1989)[88] the parameter for adjusting the pulse duration is dependent on the time of thermal relaxation of the tissue. Therefore, the time of thermal relaxation may be made compatible by reducing the duration of the laser pulse,[89] while the emission of shorter pulses could minimize the occurrence of thermal damage to the irradiated surface.[90] That is to say; the time of thermal relaxation describes the time required for the heat to propagate through the irradiated surface up to the length of the optical penetration. This is considered important when the intention is to cause localized thermal damage, with little effect on the adjacent structures.

The definition of optical penetration depth is the inverse of the optical absorption coefficient such as $z_{optical} = 1 / \alpha$.

The time of thermal relaxation is obtained by equaling the optical penetration depth L to the thermal penetration depth (z_{therm}) described in equation 2.

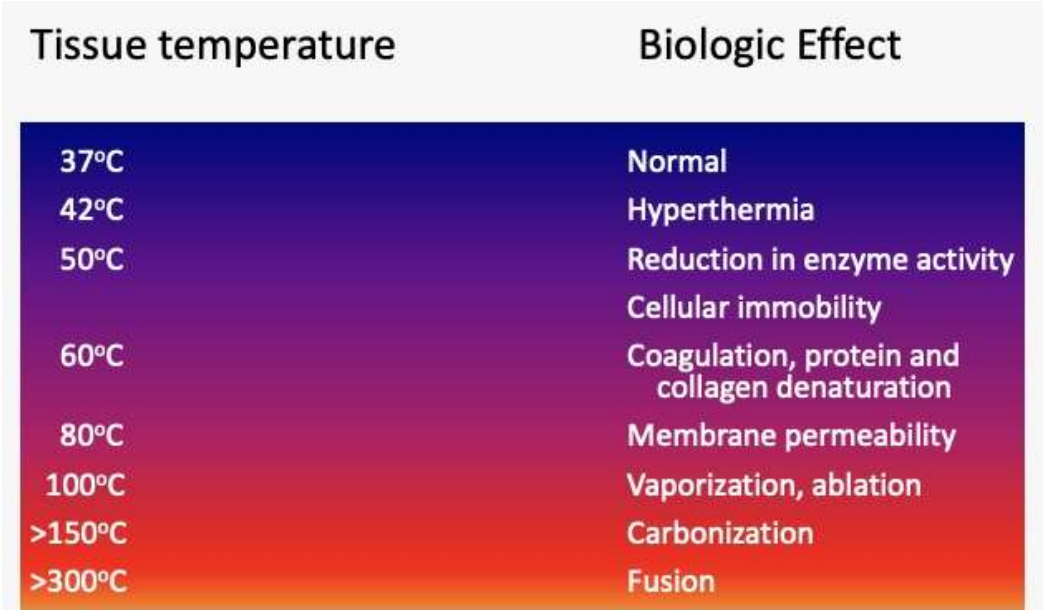
$$\tau_{therm} = \frac{1}{4k\alpha^2} \qquad (2)$$

The absorption coefficient (α) is the optical absorption of the target tissue, k is its thermal diffusivity and τ_{therm} is the time of thermal relaxation and during thermal decomposition, the τ_{therm} measures the thermal susceptibility of the tissue. Therefore, according to the theory of photothermolysis, for laser pulse duration shorter than the time of thermal relaxation ($\tau_{pulse} < \tau_{therm}$) the heat does not diffuse beyond the distance given by the optical penetration depth L; For pulse durations longer than the time of

thermal relaxation ($\tau_{pulso} > \tau_{therm}$), the head can diffuse for a multiple of the optical penetration depth; that is to say, it is possible for thermal damage to occur in the adjacent tissue.[40, 62]

With the change in the parameters of pulse duration and fluence, various effects will be promoted, ranging from ablation through to thermal lesions with an inflammatory response. The effect achieved is dependent on the increase in temperature in the target tissue and in the surrounding tissues. The thermal effects that reach temperatures of 30° C may result in dilatation and activation of the inflammatory cascade. At the temperature of 60°C we have the process of denaturation of proteins and DNA, without any vaporization of the subjacent tissue, in which, as a consequence of this process, there will be cellular loss necrosis. Above 100° C cellular there will be water vaporization, which will clinically result in ablation and/or carbonization of the tissue (Figure 5).[21, 62, 91]

Figure 5 - Effects of laser irradiation on biological tissue, depending on the magnitude of increase in temperature



Source: Niemz [62].

The lasers emitting in continuous mode have the potential to generate sufficient heat to cause the side effect of damaging the tissue adjacent to the excised tissue. The methods of beam delivery that interrupt this continuous beam, converting it into a series of intermittent pulses of energy result in higher peak power levels for a shorter period. The period between the pulses allow intermittent cooling of the target and adjacent tissues. Without cooling of the biological tissue, thermal damage may become irreversible.[92]

4.4 Thermal Damage

During the last few decades, there have been numerous scientific studies about the use of lasers in the treatment of oral diseases. However, that has been a controversy about the choice of parameters that would produce the desired clinical effect, without causing irreversible thermal damage to the adjacent tissues.[93]

Thermal damage is frequently considered an obstacle to histopathological analysis, especially because it changes the sectioned edges of the specimen.[50]

Some *in vitro* assessments have demonstrated the side effects of the use of diode laser at different wavelengths according to the tissue to be treated.[30, 94] In different tissues, the maximum absorption of diode laser has been demonstrated to be correlated to the interval of wavelength with absorption of hemoglobin, showing a considerable effect during the incision.[95] However, in the study presented by Goharkhay *et al.*, they reported that the zone of horizontal and vertical damage around the incisions performed with diode laser did not depend on the mean power or the mode used, or on the dimension of the fiber tip.[30]

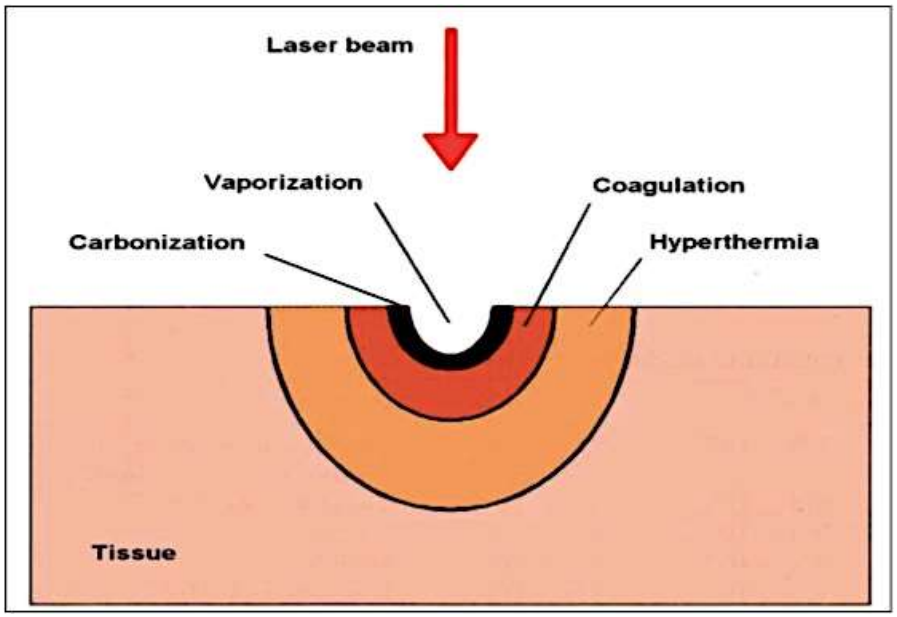
The degree of tissue damage mainly depended on the magnitude, time of exposure and placement of the heat deposited inside the tissue. The deposition of laser energy, however, is not only a function of the laser parameters, such as wavelength, power density, time of exposure of the tip and repetition rate. It also strongly depended on the optical properties of the tissue sch as the coefficients of absorption and scattering. For the description of storage and transfer of heat, the

thermal properties of the tissue are of primary importance, such as the properties of capacity for heat and thermal conductivity.(62)

Frequently, various thermal effects are induced in biological tissue, depending on the laser parameters. These effects may range from carbonization of the tissue surface through to hyperthermia for some millimeters into the tissue. In the majority of applications, however, only one specific effect is directed. Therefore, a careful assessment of the laser irradiations parameters required is necessary.(96)

It is possible to distinguish between reversible and irreversible tissue damage. Carbonization, vaporization and coagulation certainly are irreversible processes, because they induce irreparable tissue damage. Hyperthermia could, however, be a reversible or irreversible process, depending on the type of tissue and laser parameters used. The critical temperature for obtaining cell necrosis is determined by the time of exposure, which distinguishes the reversible effects from the irreversible types. Therefore, the energy of exposure, volume of exposure and duration of exposure determine the degree and extent of tissue damage.(96) The thermal effects within the tissue are illustrated in Figure 6.

Figure 6 – Schematic representation of the laser thermal effects inside a biological tissue



Source: Niemz (96).

4.5 Histological Artefacts

Biopsy is an important tool that helps with obtaining adequate representative tissue for histopathological evaluation, in order to arrive at a diagnosis later, which ranges from simple non plastic growth to malignancies.[97-100] Oral biopsies may be performed by using different techniques such as: incisional, excisional, and with the aid of punch.[101]

Selecting the biopsy technique depends on factors such as anatomic site and morphology of the lesion, and whatever the method of choice may be, the samples removed from the oral cavity are frequently small, and therefore, the possibility of producing artefacts is increased.[97, 99, 101, 102]

Incisional biopsy provides a representative sample of tissue for diagnostic purposes. It is the method of choice when the differential diagnosis includes malignancy. Its precision is relative, because by nature it does not allow the removal of the entire lesion. Unlike excisional biopsy, in which the lesion is completely removed for functional purposes, as well as for confirmation of the clinical diagnosis. This technique is appropriate for benign lesions. The size, accessibility and regional anatomy of the lesion must be considered. Small, pedunculated and exophytic lesions in accessible areas are considered appropriate for this technique.[103]

Punch biopsies are also well known for their safety and may be performed quickly.[97, 104] In this type of biopsy, a sample is removed more deeply using a cutting cylinder that passes through several layers of the skin, including the dermis, epidermis and the upper part of the subcutaneous cell tissue. However, these techniques do not provide the necessary hemostasis in highly vascularized tissue. As a result, lasers have gained popularity because they have advantages of the scalpel.[67] Nevertheless, the laser-tissue interactions may result in some changes and consequently, the presence of artefacts, particularly at the margins, such as thermal damage and coagulation that may compromise the histopathological diagnosis.[105]

To obtain an adequate histological diagnosis, the specimens must be intact and legible. In particular, integrity of the margins is most important for evaluating the infiltrative potential of pre-malignant lesions.[50] Although artefacts may be produced in

all the biopsy techniques, studies have demonstrated that there are few artefacts that are seen exclusively in laser biopsies, and these are attributed to the heat produced during the procedure.[67]

The specimens obtained with a diode *laser* have good histological legibility.[30, 106, 107] The laser configurations and their characteristics (power, wavelength, emission mode, type of fiber optic used and affinity for the target tissue) may determine the width and extension of the thermal damage caused to the tissue.[34, 108, 109]

Laser has been recommended for treating benign oral lesions, such as fibromas, papillomas, gingival hyperplasia, hemangiomas, aphthous ulcers, ankyloglossia and oral frenulum.[67, 110]

The factors that determine the initial effect of laser on the tissue include the laser wavelength, power, emission mode (continuous, pulsed) and thermal properties of the tissue.[67, 111] A possible disadvantage of using laser is related to the damage that occurs at the margins of the lesion.[112, 113]

The potential damage is of a thermal nature and occurs due to coagulation of the tissue proteins, which microscopically appear as a wide band of basophilic clot, giving the epithelium and connective tissue an amorphous appearance. The epithelial cells may also appear to be detached, fusiform, hyperchromatic and undergo vacuolar degeneration, making them useless for the purpose of diagnosis, particularly of the sample size is small.[99, 105, 114, 115]

In biopsies performed by laser, thermal damage to the epithelium may make the margins incomprehensible. Moreover, marginal artefacts such as crushing and hyperchromatic nuclei may simulate dysplasia and thereby, lead to an incorrect diagnosis.[112]

Heat emitted by the laser may cause a separation and subsequent loss of the epithelium, affecting the interpretation of the margins even further.[111, 112, 119] In the findings of Makki et al., the laser biopsies preserved the capacity for interpreting invasive malignancies, but made it difficult to assess the presence or absence of dysplasia.[112] Use of configurations with lower powers of laser may help to a certain extent to reduce the possibility of dilaceration of the epithelium and its subsequent loss during tissue processing.[120]

The use of higher average power lasers, such as the diode type, depending on the irradiation conditions, can promote the heating of the biological tissue, producing histological changes in the biopsied samples such as intracellular vacuolization, cellular hyperchromatism and loss of the intracellular structure, resulting from the degree of carbonization of this tissue. Furthermore, it could produce epithelial changes, such as bubbles, gaps, erosion and some type of intraepithelial or subepithelial loss of fixation and vascular changes such as intraluminal coagulated erythrocytes, presence of vascular stasis due to aggregated erythrocytes and thrombosis or collapse of blood or lymphatic vessels.[48, 104]

If free, legible and intact margins of the excised lesion are necessary for adequate assessment and diagnosis by pathologists, they may find serious difficulties with evaluating the tissues, due to the presence of carbonized denatured, coagulated and disorganized tissues, presence of artefacts and samples of variable extension.[48]

Development of this thesis was based on this concern about maintaining margins that were safe from thermal damage, so that biopsies of benign oral lesions could be performed with diode lasers.

5 MATERIAL AND METHODS

This study was submitted to the *Institutional Review Board (IRB)* - Ethics Committee of *The University of Texas School of Dentistry at Houston*, and to the Ethics Committee on the Use of animals of the Institute for Energy and Nuclear Research (IPEN/CNEN-SP). Because this was a study using samples of animals "*ex-vivo*" acquired from commercial breeding sites, this study was exempted by both committees (Attachments 1 and 2).

The samples were discarded in compliance with the guidelines of the Protocol *Institutional Biosafety Manual* of The University of Texas School of Dentistry at Houston.[121] The above-mentioned Establishment had the samples (swine tongues) and associated residues (plastic, paper and gauze) labeled as biological residues and sent to the sector that dealt with dangerous residues to ensure they reached their final destination.

A summary of the literature review searched in the pubmed database was performed using the keywords diode lasers, thermal damage and oral biopsy (Appendix 1).

5.1 Collection of Specimens

In this study, six recently extracted swine tongues were used (24 hours post-sacrifice) and stored at a temperature between 2 and 4°C, with 100% humidity to prevent degradation of the tissue before the experiment.[28, 122-126]

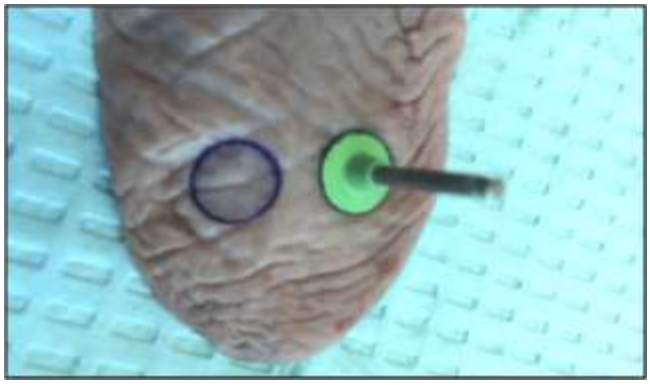
The size of specimens was previously standardized with the use of a rubber disc for polishing resin composite (Astropol Assortment Polishing Kit, Ivoclar Vivadent, Schaan, Liechtenstein). The edges were tinged with a color transfer applicator, Thompson Stick, to establish a diameter of eight mm (Figure 7).

The lasers used in this experiment were the diode type 940 nm (EPIC X™ and EPIC PRO™ Biolase, Irvine, USA) as shown in Figures 8 and 9. They were fitted with

disposable fiber of 300 micrometers and 400 micrometers in diameter respectively, used in contact mode. For specimen collection, the following items were used: Adson clinical forceps, scalpel handle and blade, marker pen, rubber disc for polishing resin, color transfer applicators and articulator paper (Figure 10).

To guarantee statistical significance, the sample calculation was made, which at first resulted in n=6 in each group, and in order to maintain a safety margin, 4 more samples were increased in each group, thus totaling n=10 specimens in each group. Ninety specimens were initially obtained, divided into nine groups cpllected with the Epic X laser with different parameters randomly distributed, and subsequently added ten more specimens performed with the Epic Pro laser, making a total of 100 specimens described in Table 1.

Figure 7 – Standardization of the samples in swine tongue with rubber disc and Thompson stick



Source: The author.

Figure 8 – Epic X diode lase (Biolase) emitting wavelength of 940 nm

Source: The author.

Figure 9 – Epic Pro diode laser (Biolase) emitting wavelength of 940 nm

Source: The author.

Figure 10 – Instruments used in biopsy procedure

Source: The author.

Table 1 - Distribution of groups according to clinical parameters most frequently used at the School of Dentistry of the University of Texas (UTHealth – Houston)

	Group	Pulse mode	Duty cycle	Pulse Repetition rate (Hz)	Average Power at display (W)	Average Power measured (W)	Power density calculated (W/cm²)	Peak power at display (W)	Pulse width	Pulse Interval (off time)
Scapel	G1 (n=10)	Control	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Epic X™ - micro pulsed diode laser (940nm)	G2 (n=10)	CW	n/a	20000	1.2	1	1415.4	1.2	n/a	n/a
	G3 (n=10)			20000	1.8	1.5	2123.1	1.8		
	G4 (n=10)	CP0	20%	20000	1.2	1	1415.4	6	10µs	40µs
	G5 (n=10)			20000	1.8	1.5	2123.1	9		
	G6 (n=10)	CP1	33%	20000	1.2	1	1415.4	3.6	100µs	200µs
	G7 (n=10)			20000	1.8	1.5	2123.1	5.4		
	G8 (n=10)	CP2	50%	20000	1.2	1	1415.4	2.4	1ms	1ms
	G9 (n=10)			20000	1.8	1.5	2123.1	3.6		
Epic Pro™ - super pulsed diode laser (940nm)	G10 (n=10)	STP	n/a	40	n/a	3.2	2547.7	80	10 µs - 100ms	0.01ms-20ms

* CW – Continuous wave
** CP0 – Initial pulsed mode
*** CP1 – First pulsed mode
**** CP2 – Second pulsed mode
***** STP – Super Thermal Pulsed mode

Source: The author.

Each group referenced for pulse mode received two different clinical surgical parameters most commonly used in the clinic of the University of Texas School of Dentistry in Houston, which were the mean powers of 1.0 W and 1.5 W. The pulse duration in Groups 4 and 5 was 10 µs, in Groups 6 and 7; it was 100 µs differently from

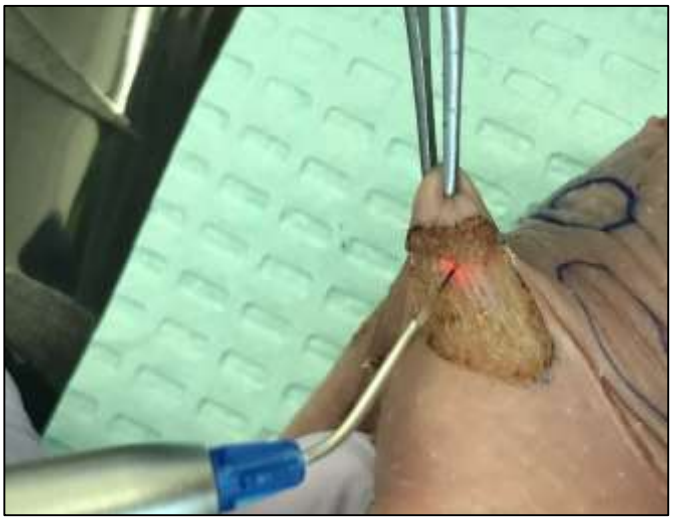
Groups 8 and 9 in which it was 1 ms and group 10 had an intrinsic equipment variation of 10 ms to 100 μs. The duty cycle ranged from 20% (Groups 4 and 5), 33% (Groups 6 and 7) and 50% (Groups 8 and 9). The Epic X diode laser was standardized by the average power according to the protocol recommended by the manufacturer, controlling its pulse width, while the Epic PRO diode laser, as its main characteristic, is an intrinsic variation, the pulse width is not controllable. Each surgical procedure was timed and registered. Each output power was verified before each surgical procedure, with the use of a power meter (PM600 Power/Energy meter, Molectron Detector Inc, Portland, OR, USA) as shown in Figure 11.

The biopsy procedures (Figure 12) with laser were performed at Houston Center for Biomaterials and Biomimetics – The University of Texas School of Dentistry, in a closed room, in accordance with the safety protocol in the Laser Safety Manual – Safety, Health, Environment & Risk Management Environmental Health and Safety Radiation Safety Program of the same institution.[127]

Figure 11 - Power meter used for checking mean output power of lasers before the procedure (PM600 Power/Energy meter, Molectron Detector Inc, Portland, OR, USA)

Source: The author.

Figure 12 - Biopsy procedure in swine tongues with Epic X diode laser



Source: The author.

5.2 Histological Preparation

The entire histological preparation and fabrication of the slides of biopsied samples were carried out at the *Department of Diagnostic and Biomedical Sciences – Oral Pathology Laboratory of The University of Texas School of Dentistry,* which followed the conventional protocol of tissue processing and staining with hematoxylin and eosin (H&E) (Attachments 3 and 4).

After collection, the biopsied samples were immediately introduced into flasks containing 10% neutral buffered formalin (StatLab, McKinney – TX, USA) to preserve the tissue in a natural state, by preventing the process of autolysis and putrefaction (Figure 13 A, B). All the specimens were section in the longitudinal position, into three equal parts thus reproducing the histological cut used for oral biopsies (Figure 14). Afterwards, all the specimens were put into cassettes and submerged in formol again (Figure 15).

The specimens were submitted to a tissue processing protocol and subsequently to the procedure of staining with Hematoxylin and Eosin. Initial tissue processing was performed in the Thermo Scientific Excelsior™ AS Tissue Processor (Thermo Fisher Scientific - Waltham, MA) that had a duration of nine hours, during which they were fixed in a 10% buffered formalin solution, twice, both times at ambient temperature for one hour, with an interval lasting 30 seconds between fixations. Afterwards, they were dehydrated and embedded in paraffin (Figures 16, 17 and 18) in accordance with the conventional international methods of histological processing.[128, 129] The paraffin blocks were sectioned by using a Leica Biosystems© microdurometer (Nussloch, Germany) in a series of 4 micrometer (µm) thick slices. Afterwards the sectioned sequence was washed and stained with Hematoxylin and Eosin (H.E) demonstrated in Figures 19 A, B and 20.

Figure 13 - A. Samples inserted into flask containing 10% buffered formalin immediately after the procedure. B. Samples collected stored in flasks containing 10% of buffered formalin

Source: The author.

Figure 14 – Longitudinal Cleavage of 3 mm thick sample with a 15 C scalpel blade.

Source: The author.

Figure 15 - Sample cleaved and stored in histological cassettes identified according to the study group

Source: The author.

Figure 16 - Automatic tissue processing in the Thermo Scientific Excelsior™ AS tissue Processor (Thermo Fisher Scientific - Waltham, MA)

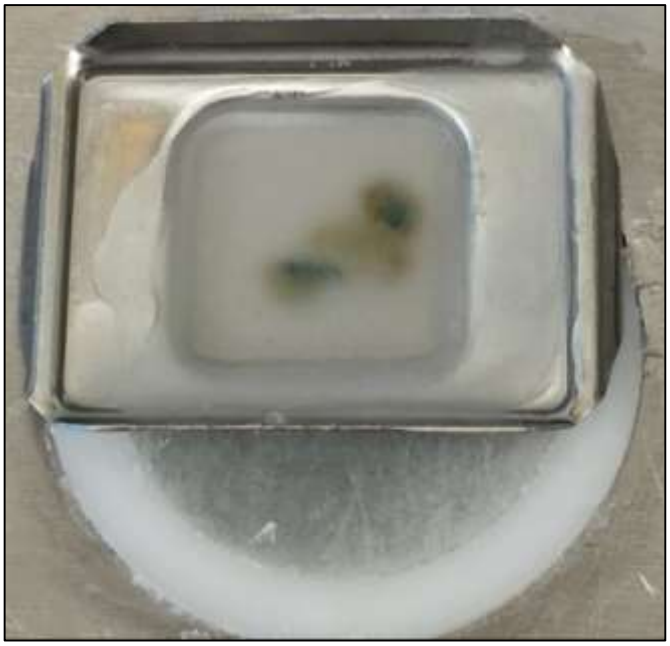
Source: ThermoFisher[1].

[1] Thermo Fisher Scientific. Waltham, MA. 2021 [citado 20 abr. 2021]. Disponível em: https://assets.thermofisher.com.

Figure 17 - Protocol Screen of tissue processing in the Thermo Scientific Excelsior™ AS tissue Processor (Thermo Fisher Scientific - Waltham, MA)

Source: The author.

Figure 18 - Embedding samples in paraffin



Source: The author.

Figure 19 – A. Microdurometer used for sectioning paraffin block; B. Sectioning paraffin block into series of 4 µm thick sections with a microdurometer (Leica Biosystems© - Nussloch, Germany)

Source: The author.

Figure 20 - Preparation of histological slide with two sequential sections in each slide

Source: The author.

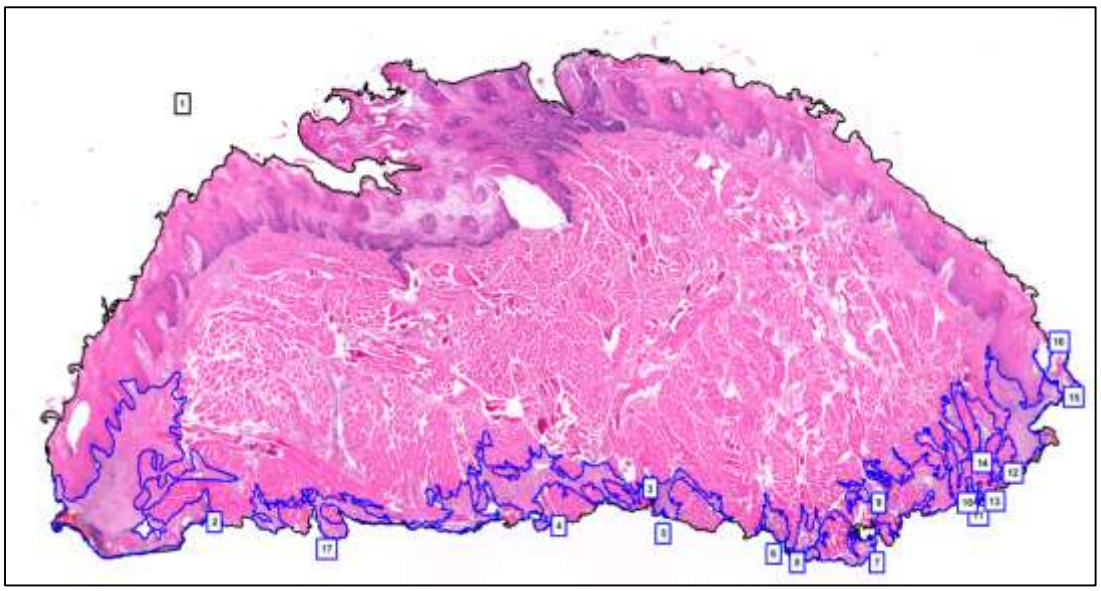
5.3 Histological Analysis

The histopathological images were evaluated under a light microscope, composed of an inverted transmission microscope (Eclipse 80i, Nikon Corporation, Tokyo, Japan),and a capture camera Andor Zyla 5.5 sCMOS (Andor Technology Company, Ltd, United Kingdom), connected to a digitizer board DS Cooled Camera Head/ DS-Qi1Mc/DS-Fi1c/DS-Ri1 (Nikon). One portion of the three pats of the samples was chosen as a standard sample in all the groups (mid-portion) shown in Figure 21. The samples were observed at 40X magnification to measure the area and depth of thermal damage to the tissue. Thermal damage was measured with use of the *NIS-Element Basic Research* software (Nikon Instruments Inc. - Melville, NY, USA) by two different systems. For the first measurement, the total area of thermal damage was described and calculated in square millimeters (mm^2) as shown in Figure 22. The second measurement was made in a line of ninety degrees starting from the base of the sample, to calculate the depth of thermal damage in micrometers (µm) shown in Figure 23. The figures demonstrate this method of analysis according to the Groups (Figure 24 A, B, C, D, Figure 25 E, F, G, H, Figure 26 I, J, K, L, Figure 27 M, N, O, P and Figure 28 Q, R).

Figure 21 - Standardization of central portion of sample to be analyzed in all study groups

Source: The author.

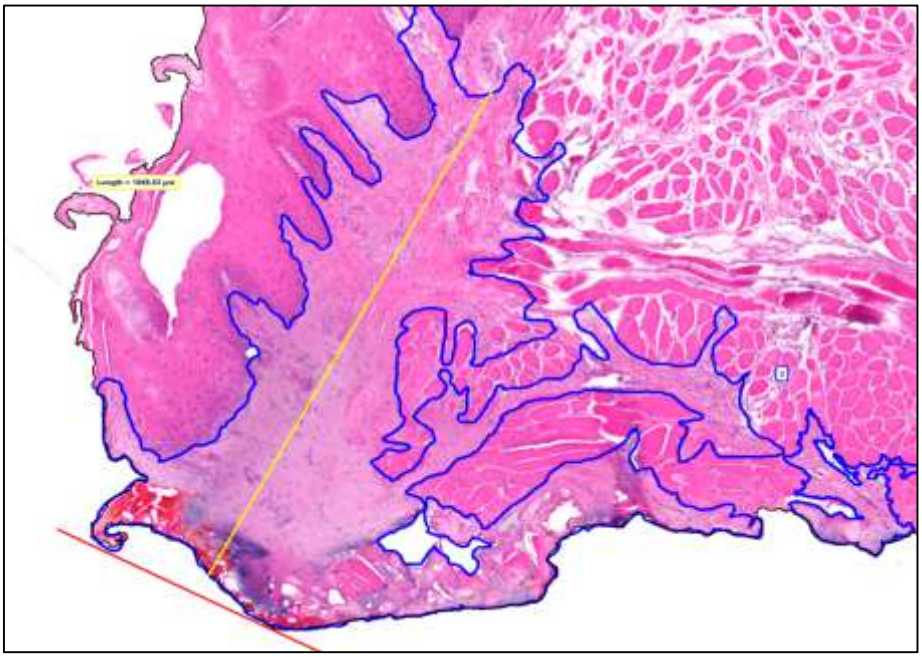
Figure 22 - Representative image of measuring total area of thermal damage in sample at 40x magnification



Source: The author.

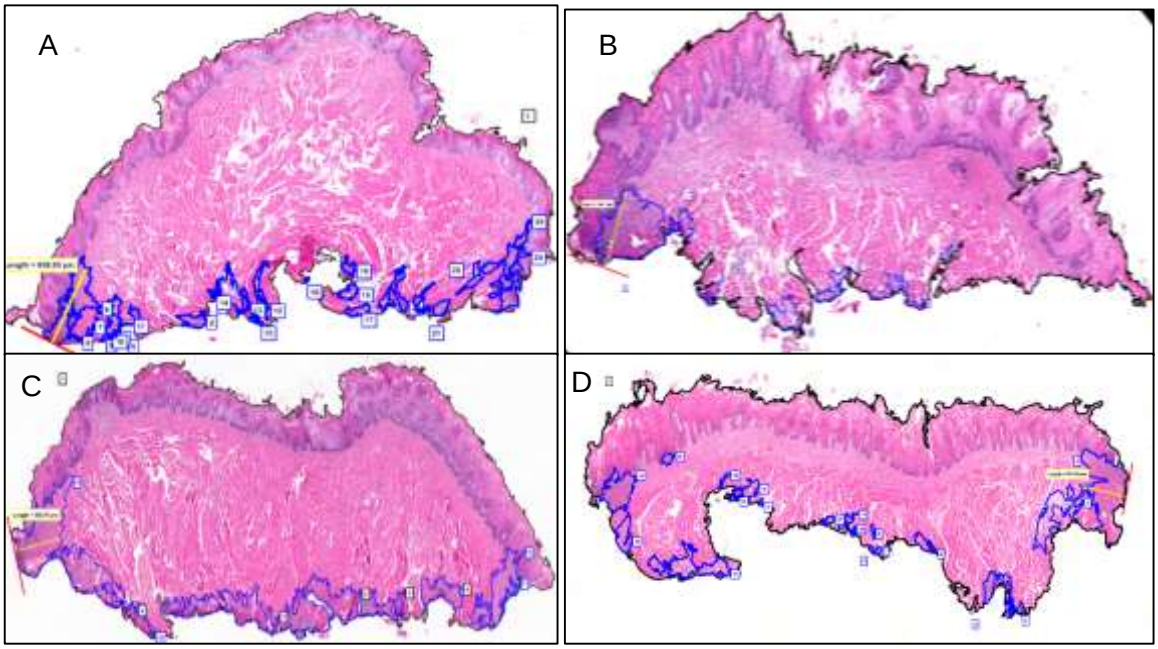
All the samples were assessed by a previously calibrated oral pathologist, in a blind study, to determine the zones of protein denaturation by excision with diode laser. Figure 23 illustrates this method of analysis per group.

Figure 23 - Representative image of measuring total depth of thermal damage in the sample (details in yellow) with digital zoom at 40x magnification



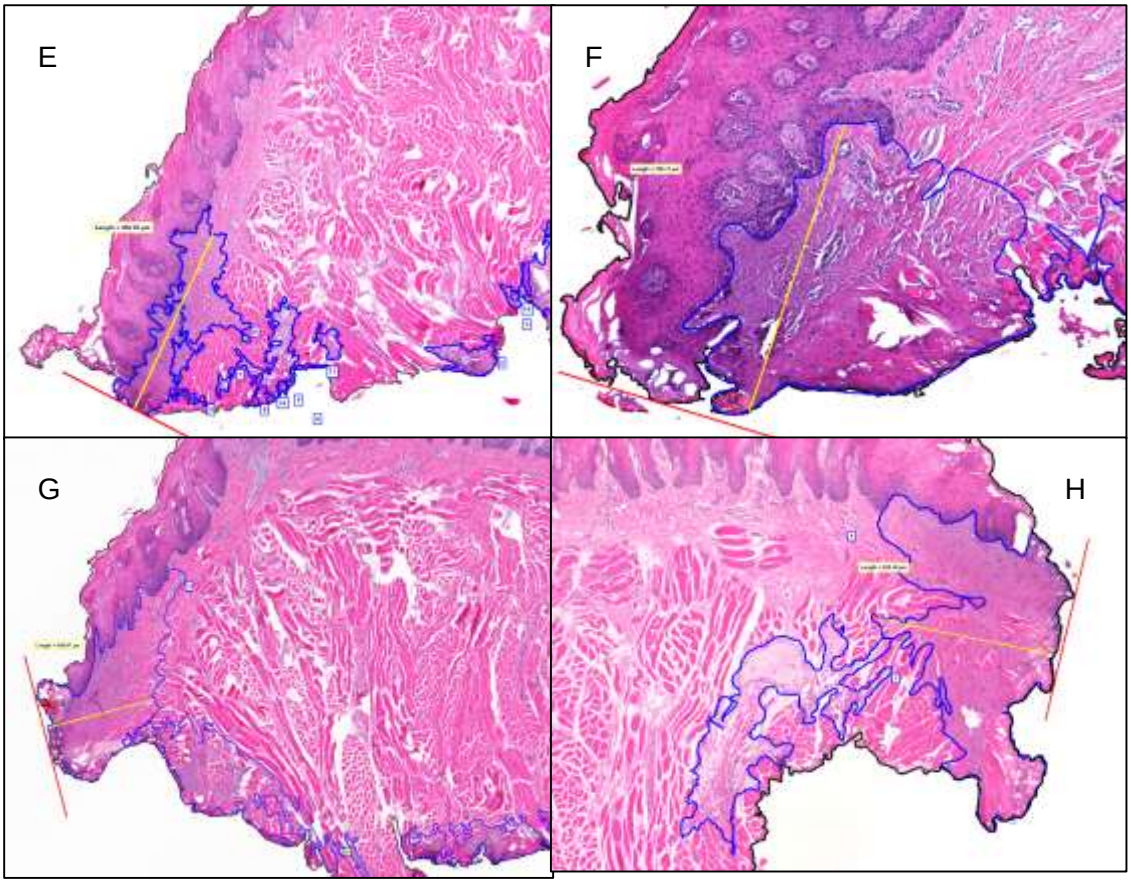
Source: The author.

Figure 24 – A. Image captured of total area of thermal damage performed with micro pulsed diode laser in continuous emission mode 1 W. B. Image captured of area of thermal damage performed with diode laser in the pulsed emission mode with power of 1 W and pulse width of 10 µs. C. Image captured of area of thermal damage performed with diode laser in the pulsed emission mode with power of 1 W and pulse width of 100 µs. D. Image captured of area of thermal damage performed with diode laser in the pulsed emission mode with power of 1 W and pulse width of 1 ms. All Images were captured at 40x magnification.



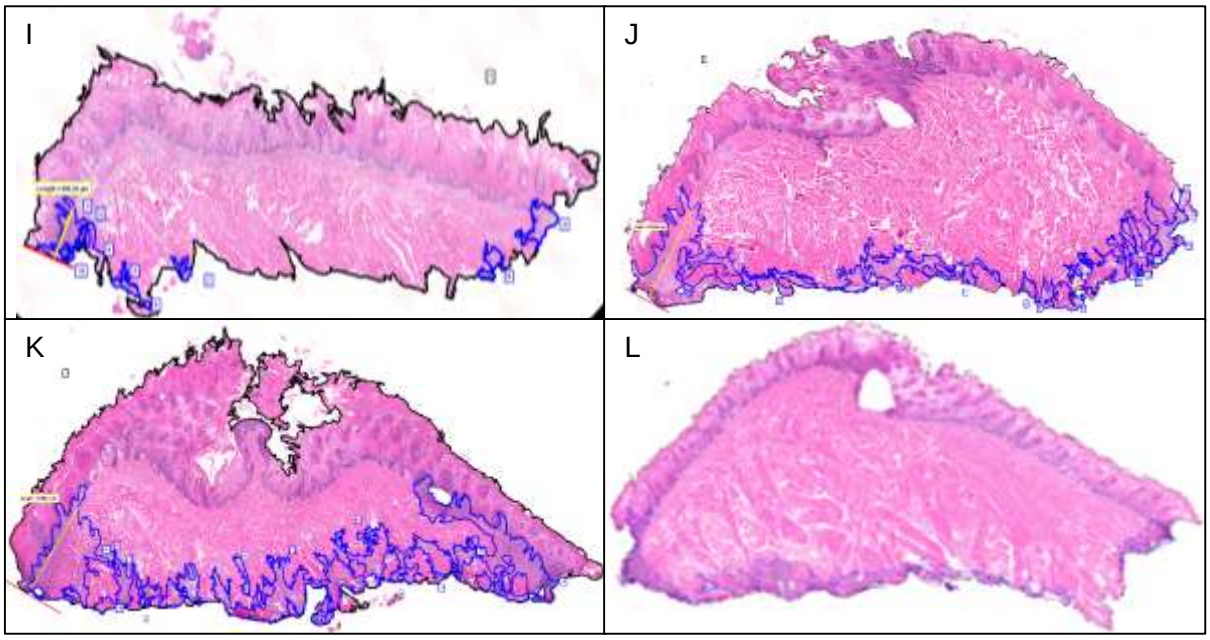
Source: The author.

Figure 25 – E. Image captured of total depth of thermal damage performed with micro pulsed diode laser in continuous emission mode 1 W. F. Image captured of total depth of thermal damage performed with diode laser in pulsed emission mode with power of 1 W and pulse width of 10 µs. G. Image captured of total depth of thermal damage performed with diode laser in pulsed emission mode with power of 1 W and pulse width of 100 µs. H. Image captured of total depth of thermal damage performed with diode laser in pulsed emission mode with power of 1 W and pulse width of 1 ms. All Images were captured at 40x magnification.



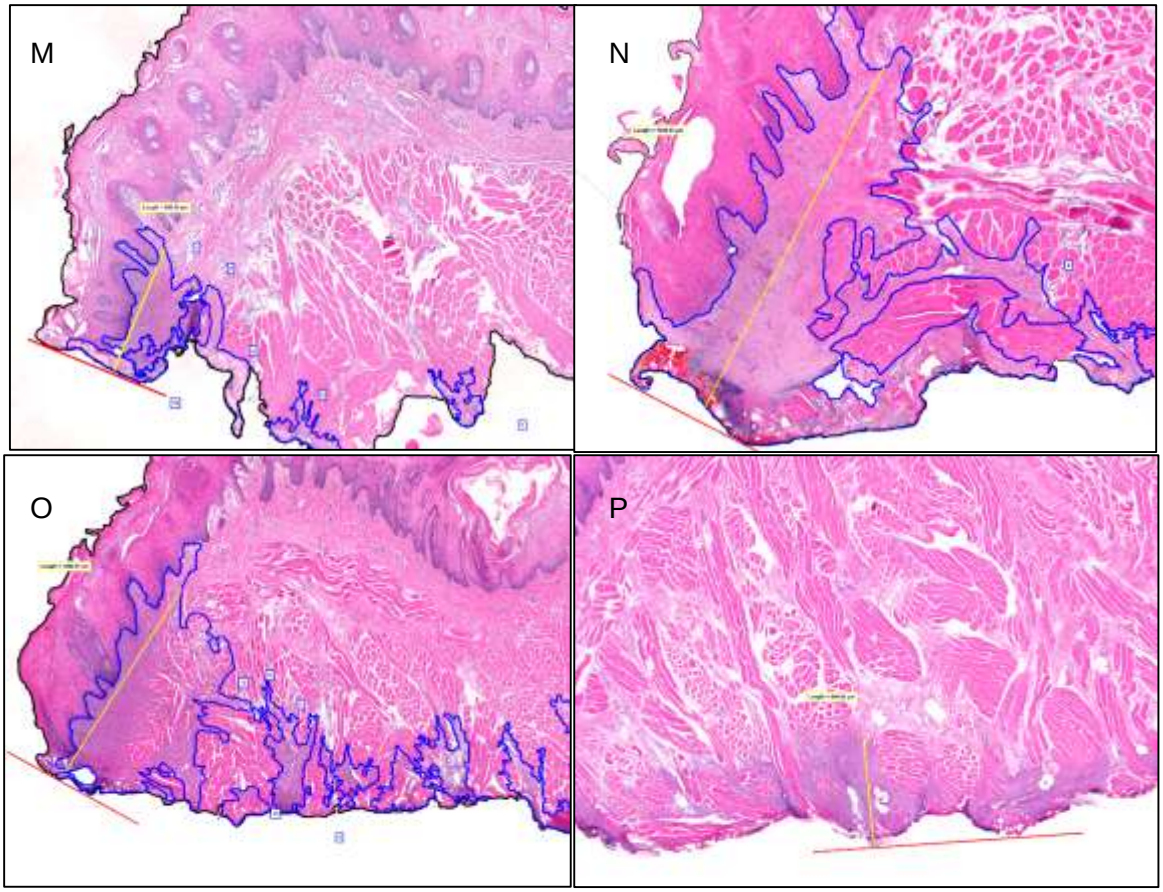
Source: The author.

Figure 26 – I. Image captured of total area of thermal damage performed with micro pulsed diode laser in continuous emission mode 1.5 W. J. Image captured of area of thermal damage performed with diode laser in pulsed emission mode with power of 1.5 W and pulse width of 10 µs. K. Image captured of area of thermal damage performed with diode laser in pulsed emission mode with power of 1.5 W and pulse width of 100 µs. L. Image captured of area of thermal damage performed with diode laser in pulsed emission mode with power of 1.5 W and pulse width of 1 ms. All Images were captured at 40x magnification.



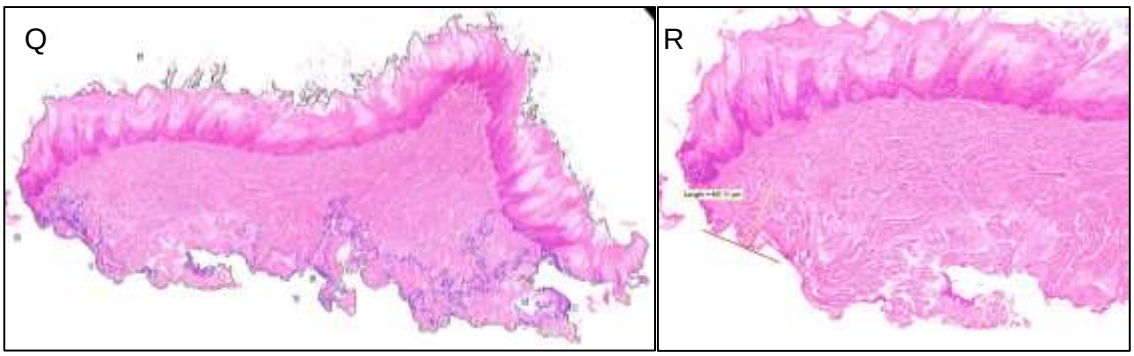
Source: The author.

Figure 27 – M. Image captured of total depth of thermal damage performed with micro pulsed diode laser in continuous emission mode 1.5 W. N. Image captured of depth of thermal damage performed with diode laser in pulsed emission mode with power of 1.5 W and pulse width of 10 µs. O. Image captured of depth of thermal damage performed with diode laser in pulsed emission mode with power of 1.5 W and pulse width of 100 µs. P. Image captured of depth of thermal damage performed with diode laser in pulsed emission mode with power of 1.5 W and pulse width of 1 ms. All Images were captured at 40x magnification.



Source: The author.

Figure 28 – Q. Image captured of total area of thermal damage performed with super pulsed diode laser with power of 3.2 W. R. Image captured of depth of thermal damage performed with super pulsed diode laser with power of 3.2 W. All Images were captured at 40x magnification.



Source: The author.

5.4 Statistical Analysis

The statistical analyses were performed using the software package SPSS, V20.0 with a level of significance of 5%. The distribution of numerical variables was investigated by means of histograms and Box plots, and by the Shapiro-Wilk normality test. The distributions observed were asymmetrical and these measurements were described by medians and quartiles, minimum and maximum values. The non parametric Kruskall-Wallis tests were applied to compare the study groups relative to the measurements of area of thermal damage, depth of thermal damage and time of excision. The differences were found in the Dunn multiple comparison tests with Bonferroni correction. The p-value table was used to localize the differences in the multiple comparisons, in which the significant value was $p < 0.05$. As there are diverse tests being applied with the same objective, we adjusted the values to maintain an overall level of significance of 5%. Therefore, the adjusted values refer to the results of comparisons between each pair of Groups, adjusted for the multiple comparisons.

6 RESULTS

6.1 Area of Thermal Damage

The Control Group (scalpel) showed no thermal damage. In the Experimental Groups with laser (G2-G10), the total area of damage observed (mm^2) was lower in Group G3 (CW - 1.5W) with median of 0.91 mm^2 and $p < 0.05$ (Table 2) (Figure 29). When the multiple comparison tests were applied and the overall level of significance was controlled, the median of the damaged area in Group 3 was lower than the adjusted medians of G7 (1.5W, work cycle of 33%), and G9 (1.5W, work cycle of 50%). No statistically significant differences were observed in the other Groups.

Table 2 – Total thermal damage area (mm^2) of specimens according to the study group

Groups	Minimum	1st quartile	Median	3rd quartile	Maximum	n
G2	0.46	1.03	1.09	1.54	1.98	10
G3	0.40	0.53	0.91*	1.11	2.60	10
G4	0.71	0.91	1.22	1.86	2.99	10
G5	0.93	1.26	1.37	1.66	3.93	10
G6	0.34	1.20	1.53	1.72	1.90	10
G7	1.32	1.52	1.93*	2.45	3.71	10
G8	0.73	1.09	1.50	2.42	3.37	9
G9	0.77	1.40	1.97*	3.50	3.97	10
G10	0.57	0.90	1.08	1.74	2.76	9

Kruskal-Wallis Test p = 0.009*

*G3 differed significantly when compared with G7 and G9; G1, Control Group was excluded from the evaluation of thermal damage because no laser had been used in it.

Source: The author.

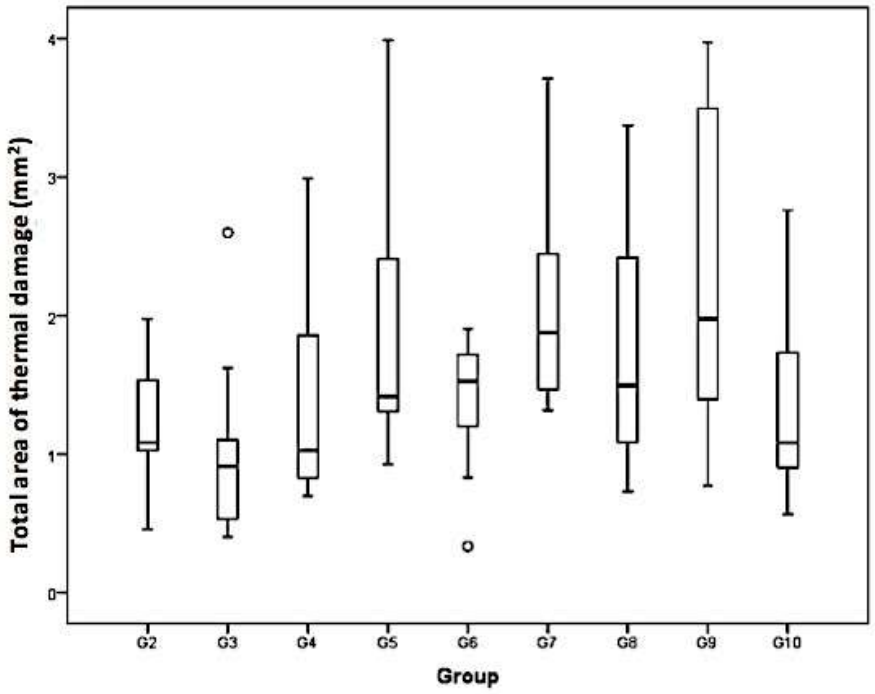
Tabela 3 – Multiple comparisons between groups regarding the total area of thermal damage

Pairs	p-value	adjusted p-value
G2 x G3	0.345	>0.999
G2 x G4	0.636	>0.999
G2 x G5	0.310	>0.999
G2 x G6	0.446	>0.999
G2 x G7	0.009	0.307
G2 x G8	0.216	>0.999
G2 x G9	0.019	0.684
G2 x G10	0.893	>0.999
G3 x G4	0.156	>0.999
G3 x G5	0.050	>0.999
G3 x G6	0.088	>0.999
G3 x G7	<0.001	0.013*
G3 x G8	0.031	>0.999
G3 x G9	0.001	0.036*
G3 x G10	0.292	>0.999
G4 x G5	0.587	>0.999
G4 x G6	0.773	>0.999
G4 x G7	0.031	>0.999
G4 x G8	0.437	>0.999
G4 x G9	0.061	>0.999
G4 x G10	0.745	>0.999
G5 x G6	0.800	>0.999
G5 x G7	0.106	>0.999
G5 x G8	0.803	>0.999
G5 x G9	0.183	>0.999
G5 x G10	0.393	>0.999
G6 x G7	0.062	>0.999
G6 x G8	0.620	>0.999
G6 x G9	0.113	>0.999
G6 x G10	0.544	>0.999
G7 x G8	0.186	>0.999
G7 x G9	0.776	>0.999
G7 x G10	0.015	0.550
G8 x G9	0.296	>0.999
G8 x G10	0.282	>0.999
G9 x G10	0.032	>0.999

*p value < 0.05 after adjustment for multiple comparisons between each pair of Groups.

Source: The author.

Figure 29 - Representative graph of total area of thermal damage per study group



Source: The author.

6.2 Depth of Thermal Damage

The Control Group (G1) showed no thermal damage. In the Experimental Groups (G2-G10), the depth of thermal damage (μm) in the samples showed no evidence of statistical differences between the Groups (p=0.125) (Table 4) (Figure 30).

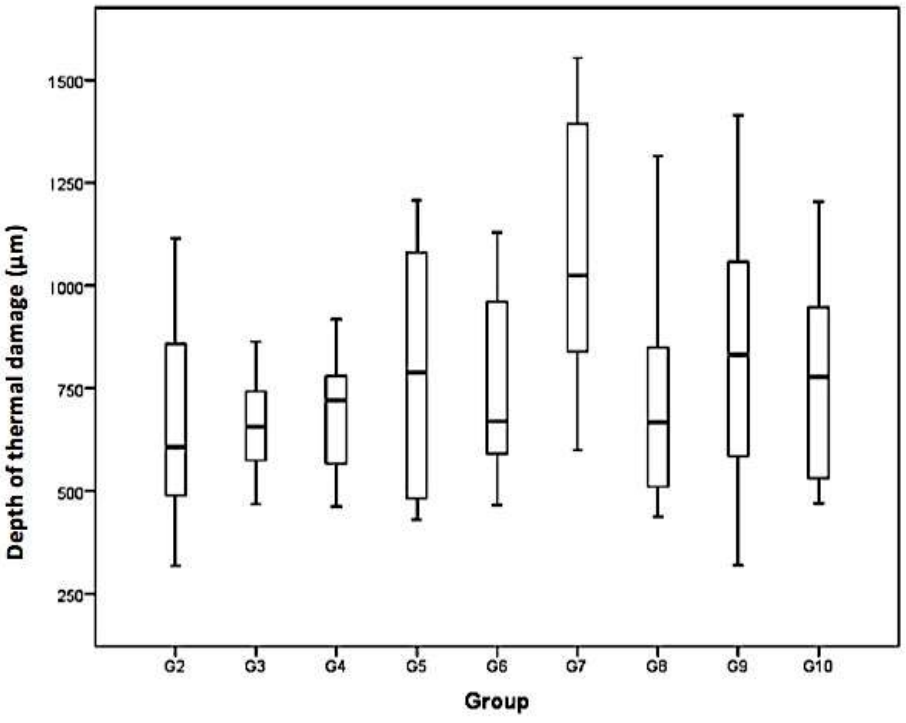
Table 4 - Depth of thermal damage (m) of specimens according to Study Group No statistical significance was detected between the Groups. G1, Control Group was excluded from the evaluation of thermal damage because no laser had been used in it

Groups	Minimum	1st quartile	Median	3rd quartile	Maximum	n
G2	317.6	489.4	606.4	858.1	1114.7	10
G3	468.0	574.5	655.8	742.8	863.2	10
G4	462.0	566.8	720.4	779.9	918.3	10
G5	429.7	482.5	789.6	1080.0	1206.2	10
G6	465.1	590.8	669.3	960.4	1129.8	10
G7	599.5	839.8	1024.9	1393.5	1555.0	10
G8	437.3	510.5	666.4	849.7	1397.9	9
G9	319.0	584.6	831.1	1057.5	1414.0	10
G10	470.6	530.5	778.8	947.1	1203.6	10

Kruskal-Wallis Test p = 0.125

Source: The author.

Figure 30 - Depth of thermal damage per study group

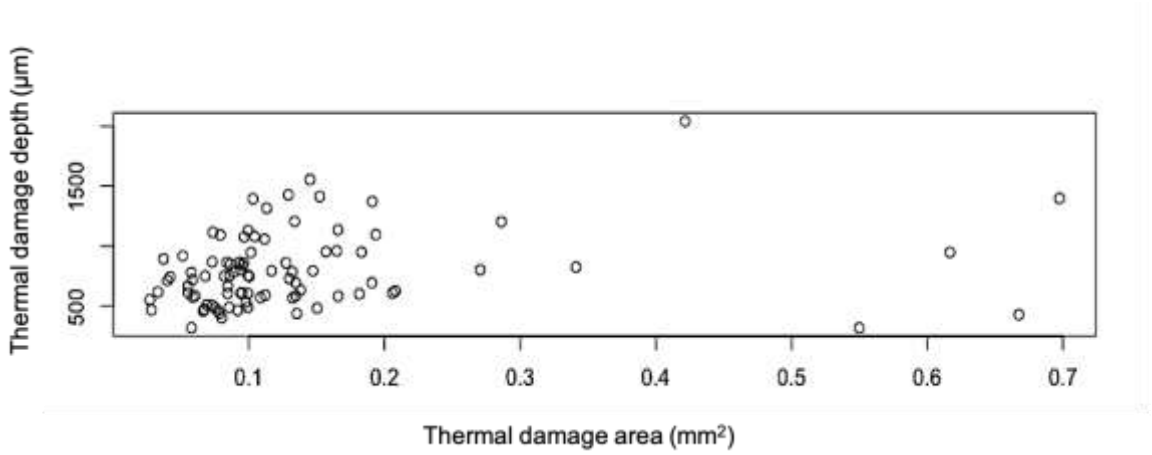


Source: The author.

6.3 Correlation of the Area and Depth ff Thermal Damage

The total area of thermal damage (mm^2) ranged from 0,91-1,97 mm^2 (Table 2) and the depth of thermal damage ranged from 606-1024 µm (Table 3). A positive correlation was observed between depth (µm) and area (mm^2) when thermal damage was assessed (Figure 31).

Figure 31 - Positive correlation between depth (µm) and area (mm^2) caused by thermal damage



Source: The author.

6.4 Time of Excision

We point out that the median time of excision in the super pulsed diode laser group (G10) was comparable with that of the Control Group (G1). The medians of the times of excision in G1 and G10 were significantly lower ($p < 0.001$) than the medians of the micro pulsed laser Groups (G2-G9) (Table 5) (Figure 32).

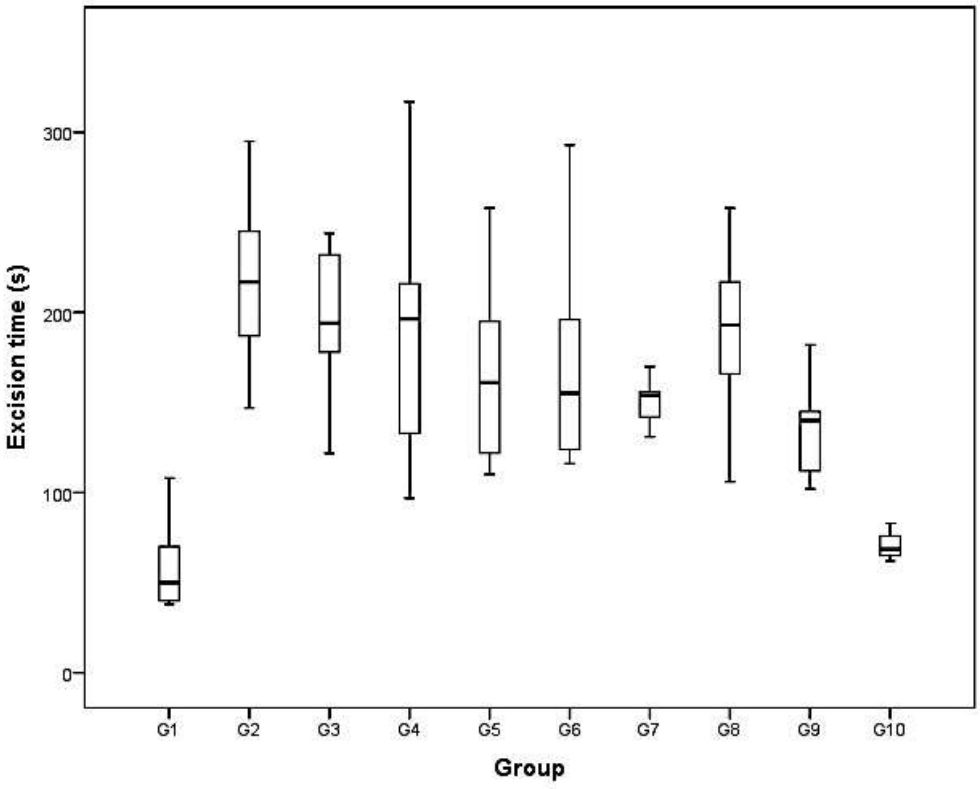
Table 5 – Time of excision (s) of specimens according to Study Group. Please note that G1 (scalpel) and G10 (super pulsed diode laser) differed statistically from all the other Groups ($p < 0.05$)

Groups	Minimum	1st quartile	Median	3rd quartile	Maximum	n
G1	38	42	50 *	68	108	10
G2	187	201	238	270	350	10
G3	122	179	194	227	244	10
G4	97	147	197	213	317	10
G5	110	122	161	194	258	10
G6	116	130	155	190	293	10
G7	131	142	155	158	306	10
G8	106	138	193	248	309	9
G9	110	122	142	150	205	10
G10	62	66	69 *	75	83	9

Kruskal-Wallis Test p=0.001*

Source: The author.

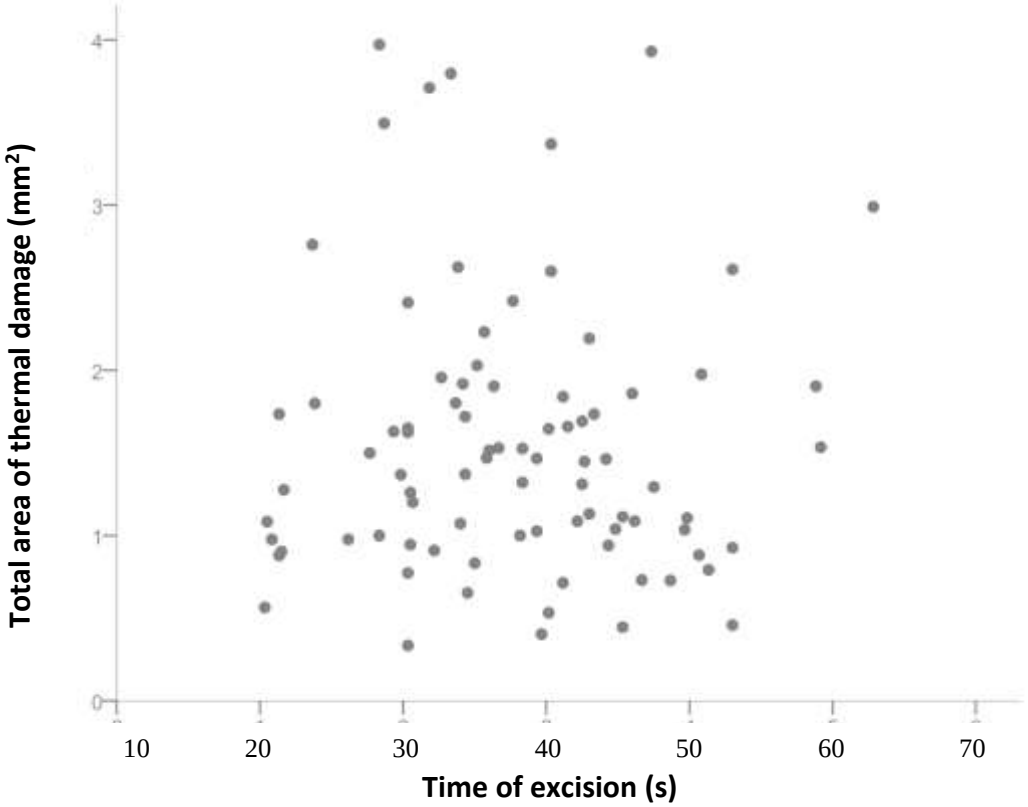
Figure 32 – Time of excision per study group



Source: The author.

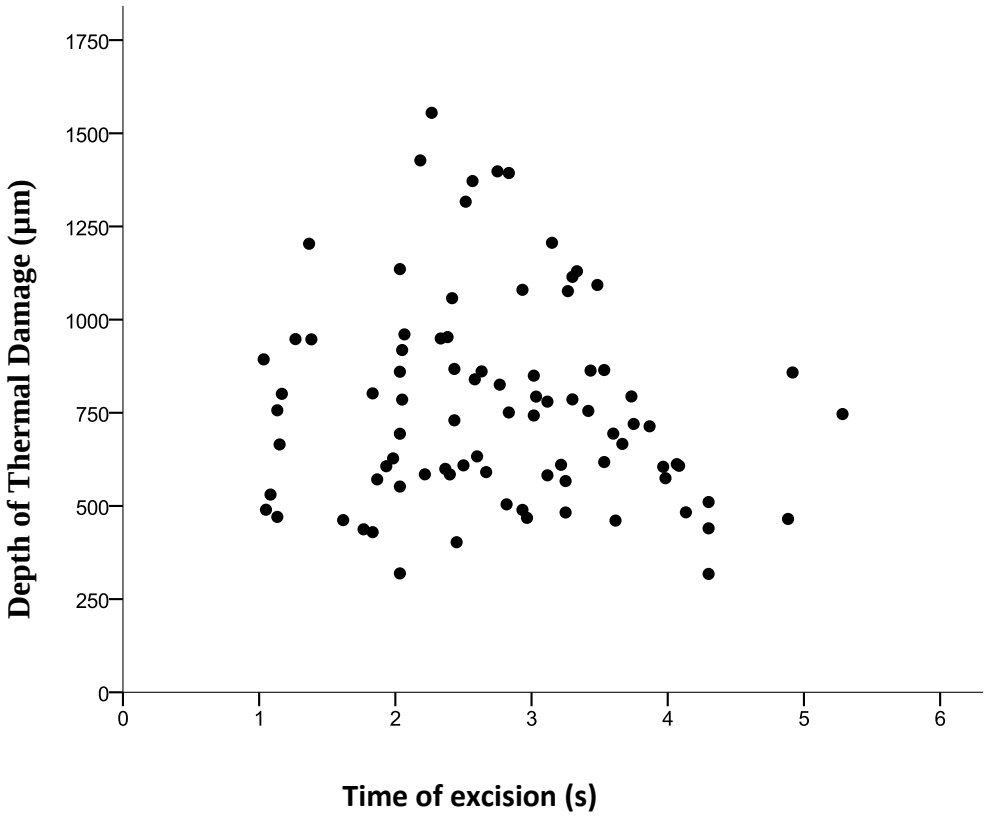
We investigated the correlations between the time of excision and the measurements of total area of thermal damage and depth of thermal damage (Figures 33 and 34) and found coefficients indicating the absence of important correlation (r = -0.036 p = 0.740 for area of thermal damage and r = -0,092 p = 0.399 for depth of thermal damage).

Figure 33 - Dispersion representing correlation between time of excision and total area of thermal damage



Source:The author.

Figure 34 - Correlation between time of excision and depth of thermal damage



Source: The author.

7 DISCUSSION

Various studies have demonstrated that the CO_2, Er: YAG, Nd: YAG and diode lasers could be used for performing biopsies on oral soft tissues without causing significant thermal damage and making it difficult to achieve histopathological diagnosis.[23, 130] However, very few have evaluated and compared the variables such as thermal damage ad times of excision produced by micro and super pulsed diode lasers, which are the most common surgical lasers used in contemporary clinical dentistry.[23, 50, 53, 131, 132]

To fill this gap, in the present study, two diode lasers with different combinations of power, duty cycle, and pulse duration were evaluated. The results of this study supported the view that different parameters could affect the soft tissue responses in various ways, such as thermal damage and time of excision. Furthermore, this study showed a positive correlation between the area and depth of tissue affected when the total thermal damage was measured. Therefore, our discussion reflects the results of both methodologies when making reference to thermal damage.

In the present study, pig tongues were used, because they are histologically and physiologically similar to human tongues.[28, 133, 134] The researchers selected a diameter of 8 mm as the standardized sample size for two main reasons. Laser surgery is not recommended for any lesion with a size of less than 5 mm, due to the high probability of artefacts induced by heat interfering in the microscopic assessment.[27, 50] Secondly, 8 mm represents the mean size of the majority of common, simple excisional oral biopsies, with the addition of 1 mm at the margin of healthy peripheral tissue for adequate histological analysis. Moreover, the larger sample size is more adequate for handling in an *ex-vivo* environment. For example, fibromas, which are the most frequently found benign tumors (53.3%) of the oral cavity, range between 5 - 8 mm in size. [135-139] The most common localizations for these slow growing lesions are the oral mucosa along the occlusal line and in the tongue edge due to occlusion trauma.[135, 136] The treatment is surgical excision and recurrence is rare. According to the literature, it is advisable to use laser as the surgical tool, and leave a halo of 0.5 – 1 mm of healthy tissue at the periphery of the pathology. This preserves the morphological and structural characteristics of the specimen collected, and allows a

legible and reliable diagnosis to be made.[23] According to this study, the deepest area of thermal damage within the specimen, on an average, ranged from 606 - 1024 µm. This supported the suggestion of Romeo *et al*, of leaving a band of healthy tissue at the periphery of the margins of the lesion, to compensate for any artefacts induced by heat.[133]

In this study, it was observed that the micro pulsed diode laser G3 (CW, 1.5W) produces a smaller total area of thermal damage (mm^2) when compared with all the groups, in absolute numbers, however, the statistical differences were only identified in the comparison of the analysis of multiple comparisons among Groups G3 x G7 x G9 (Table 3). The Groups with the largest area of thermal damage (mm^2) were G9 (1.5W, work cycle of 50%) and G7 (1.5W, work cycle of 33%), whereas the smallest damage was identifies as being G3 (CW, 1.5W) followed by G10 (super pulsed laser, 3.2 W). These observations may be partially explained by a phenomenon called the “hot spot” effect in combination with the pulse duration of the laser. The hot spot effect is caused when a target tissue absorbs the laser energy, leading to denaturation of the protein and carbonization of the tissue. This leads to an accumulation of carbonization debris on the tip of the fiber, which continues to overheat by several hundreds of degrees, and promotes the cut and coagulation of the tissue.[140] With diode lasers the recommendation is to perform constant removal of this accumulation of carbonized tissue from the tip of the laser. Although removal of this aggregated debris is necessary, the clean tip has a reduced capacity for absorbing the heat, allowing it to be transferred directly to the target tissue at a deeper level of penetration. As this cycle of accumulation of carbonization residues and continual cleaning occurs, a fluctuation in the depth of thermal penetration and cutting efficiency at the same site is observed. In addition to this variation in heat penetration, the laser pulse duration is another important factor that could impact negatively on the tissue response to uncontrolled thermal damage. The pulse duration refers to the time in which the biological tissue is exposed to irradiation with laser. The longer the time interval, the more laser-tissue interaction will be observed [53, 141] Moreover, the quantity of power (peak vs. average) may dictate the degree of interaction at the surgical site. The peak power and maximum optical power that a laser can produce to interact with the target tissue within a specific period of time, corresponds to the energy delivered during the short duration of each laser pulse. The mean power is the energy transferred to the target tissue per

unit of time; or for pulsed lasers, it is the product of energy per pulse and the frequency of repetition of the pulses. Irrespective of the parameter of power sed, it is important to balance the thermal effects within a tissue, by allowing the thermal energy to dissipate. This cooling time is known as thermal relaxation.[142] An adequate laser irradiation protocol must allow the reduction of damaging side effects generated by heat, by balancing the power and pulse duration. Pulse duration exceeding 1 µs exhibited perceptible thermal effects on the oral soft tissue.[62] In the present study, Groups G7 and G9 with longer pulse duration (100 µs and 1 ms) and a high peak power (5.4 W and 3.6 W) resulted in a larger area of heat transmission into the specimens. In contrast, the super pulsed diode laser (G10) had a pulse duration of longer than 1 µs (10 µs – 100 ms) and a high peak power (80 W), but caused less thermal penetration. This could be explained by the new leading edge technology of this super pulsed diode laser (Epic Pro™), in which a mechanism of controlled thermal feedback is installed to read the temperature of the tissue and adjust its energy at the fiber tip during the time of excision. The purpose of this advanced system is to avoid overheating the tissue and improve the cutting efficiency.[44]

The findings of our study showed good congruence with those of Wilder-Smith *et al*, 1997; who observed that the incisions made with a CO_2 laser in super pulsed mode (pulse duration of 300 µs, peak power of 60 - 100 W and mean power of 0.7-1.2W) showed a significant reduction in thermal damage side effects in comparison with the continuous mode.[37] Our results also corroborated the finding of Romanos *et al,* because the time of removal of the biopsied tissues obtain with the super pulsed diode laser (G10) was significantly faster than it was in the other Groups.[44] Furthermore, the cutting efficiency of the super pulsed diode laser was noted to be comparable with that of a scalpel.

To the best of the authors' knowledge, this is the first study to evaluate the effects of different micro and super pulsed diode lasers on the integrity of tissue specimens and their cutting efficiency. Therefore, the study provides a body of inestimable basic [scientific] information that will be useful for future studies. Furthermore, this study provides useful information that may guide doctors in performing laser excision of simple surgical specimens.

Relative to limitations of the study, in spite of the many similarities to human tissues, the use of swine tongue mucosa is not the mucosa of the human tongue. The authors recognize the possibility that there might be some variations in the results obtained between the mucosa of real human tongue [and the tissue used in the present study]. Furthermore, this study had various limitations with regard to the comparison between the new super pulsed laser and micro pulsed laser, seeing that with the latter equipment it was not possible to control the peak power and pulse width, because it presents an intrinsic variable, contrary to the super pulsed laser. Another limitation of this study was to obtain an exact specimen size in a reproducible manner. Even with the diameter of 8 mm defined, all the measurements were not always exactly precise, because the laser cut of the tissue was performed manually and this may have introduced the possibility of occurrence of small random variations in mm^2. To overcome this limitation, however, the researchers tested two methods of measuring the thermal damage (depth and area). After statistical analysis, we concluded that the measurements were positively correlated and could be applied in an independent or collective manner.

Future studies could test more irradiation parameters of the super pulsed diode lasers in comparison with the parameters of micro pulsed lasers considering that under the working conditions of this study, super pulsed diode lasers produced less thermal damage.

8 CONCLUSIONS

The super pulsed diode laser with the new thermal feedback system demonstrated a more efficient cutting capacity than the micro pulsed laser, and was comparable with the scalpel. A minimum of 1 mm of perimeter of healthy margins is recommended for appropriate microscopic analysis of the tissue sample. This “Safety Zone” will be sufficient to support the damaging side effects caused by laser heat according to the parameters described in this study. However, few clinicians are capable of substituting their existent laser by the most recent technology as it becomes available.

Furthermore, the results of this study confirmed that the diode laser can be used as an adequate surgical tool for the excision of simple, benign tumors when the appropriate irradiation parameters are used. Therefore, in view of the knowledge obtained in this *ex-vivo* study, general guidelines can be suggested for improved clinical results.

As the time of excision did not show correlation with the area and depth of thermal damage, this does not allow affirmation that the faster the incision is performed, the smaller will be the area or depth of damage.

According to the results of this study, professional clinicians using:

• conventional diode lasers may consider a continuous mode of emission instead of a work cycle of 50%;

• for the micro pulsed diode laser, the parameter of shorter pulse duration offered by the equipment, or continuous mode of emission may be selected;

• for the super pulsed diode laser, the parameter of lower cutting power offered in this new generation of lasers may be selected.

The above-mentioned guideline may reduce the damaging side effect generated by heat, and allow an adequate histological evaluation of the biopsied tissues.

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ATTACHMENT 1 – Exemption of the Animal Welfare Committee (AWC) and Institutional Review Board (IRB).

UTHealth
The University of Texas
Health Science Center at Houston
School of Dentistry

From: Dang, Auco
Sent: Monday, October 16, 2017 3:49 PM
To: Barros, Juliana <Juliana.Barros@uth.tmc.edu>
Subject: RE: FINAL PROTOCOL

Dr. Barros,

I reviewed your protocol and it does not appear that you are using any human derived samples, human derived data or human subjects for your research project and will only using pig tongues. These are purchased from a particular commercial entity and do not need AWC approval.

The pig tongues should be disposed following the guidelines from the Health and Safety office SHERM https://www.uth.edu/safety/, but otherwise, you would **not need** IRB approval https://www.uth.edu/cphs/policies/requires-review.htm .

Thank you
Auco

Ms. Auco Dang
Research Coordinator I

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Houston's Health University

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ATTACHMENT 2 – Approval from Ethic Committee on Animal Use of the Nuclear and Energy Research Institute (IPEN/CNEN).

ipen

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada **"Pode um laser de diodo micropulsado e superpulsado manter a integridade e qualidade marginal do espécime necessários para uma adequada avaliação histopatológica?"**, registrada com o nº **241/19,** sob a responsabilidade de **Denise Maria Zezell, NÃO UTILIZA** animais pertencentes ao filo Chordata, subfilo Vertebrata, de acordo com a Lei nº 11.794 de 8 de outubro de 2008, do Decreto nº 6.899 de 15 de julho de 2009 com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e **SIM,** tecidos oriundos de estabelecimentos comerciais (açougues), portanto está **ISENTA** de avaliação pela CEUA-IPEN - Comissão de Ética no Uso de Animais do Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN-SP).

We certify that the proposal titled **"Can a Micropulsed and Superpulsed Diode Laser Maintain the Marginal Integrity and Quality of a Specimen Needed for adequated Histological Evaluation?"**, registration number **241/19**, under the responsibility of **Denise Maria Zezell, DOES NOT USE** animals belonging to the phylum Chordata, subphylum Vertebrata, in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), **BUT** samples obtained commercially (butchers), therefore **IT DOES NOT REQUIRE EVALUATION** by the CEUA-IPEN - Ethic Committee on Animal Use of the Nuclear and Energy Research Institute (IPEN/CNEN-SP).

São Paulo, 23 de Maio de 2019.

Patrick Jack Spencer
Coordenador da CEUA-IPEN

Instituto de Pesquisas Energéticas e Nucleares (IPEN-CNEN/SP)
Centro de Biotecnologia
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Telefone: (011) 3133-9696 – E-mail: ceuaipen@ipen.br

ATTACHMENT 3 – Histology Processing Protocol of Department of Diagnostic and Biomedical Sciences – Oral Pathology Laboratory – University of Texas School of Dentistry at Houston.

UTHealth
The University of Texas
Health Science Center at Houston

School of Dentistry
Department of Diagnostic and Biomedical Sciences
Oral Pathology Laboratory

Program # 1 – Routine Overnight – Duration 9:04

Reagent	Temperature	Time	Vacuum	Drain Time
Formalin	Ambient	1:00	Off	30s
Formalin	Ambient	1:00	Off	60s
Dehydrant	Storage (=Ambient)	:40	On	30s
Dehydrant	Storage	:30	On	30s
Dehydrant	Storage	:30	On	30s
Dehydrant	Storage	:30	On	30s
Dehydrant	Storage	:30	On	30s
Dehydrant	Storage	:30	On	60s
Xylene	Storage	:30	On	30s
Xylene	Storage	:30	On	30s
Xylene	Storage	:40	On	120s
Paraffin	Storage (=62°C)	:40	On	120s
Paraffin	Storage	:40	On	120s
Paraffin	Storage	:40	On	120s

713.486.4413 Office | 713.486.4407 Lab
7500 Cambridge Street, Suite 6110
Houston, Texas 77054
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ATTACHMENT 4 – Hematoxylin and Eosin Staining Processing Protocol of Department of Diagnostic and Biomedical Sciences – Oral Pathology Laboratory - University of Texas School of Dentistry at Houston.

UTHealth
The University of Texas
Health Science Center at Houston

School of Dentistry
Department of Diagnostic and Biomedical Sciences
Oral Pathology Laboratory

H&E Staining Procedure Using Automatic Stainer (broke down by staining jars):

1. Place slide in stainer clip and place on stainer chain at beginning of heater area.....5 minutes
2. Xylene.....30 seconds
3. Xylene.....30 seconds
4. Xylene.....30 seconds
5. Xylene.....30 seconds
6. Xylene.....30 seconds
7. Xylene.....30 seconds
8. 100 % Alcohol.....30 seconds
9. 100 % Alcohol.....30 seconds
10. 100 % Alcohol.....30 seconds
11. 95 % Alcohol.....30 seconds
12. diH2O.....30 seconds
13. Hematoxylin 560 MX.....30 seconds
14. Hematoxylin 560 MX.....30 seconds
15. Hematoxylin 560 MX.....1 minute
16. Hematoxylin 560 MX.....1 minute
17. diH2O.....30 seconds
18. Define MX-aq*.....30 seconds
19. diH2O.....30 seconds
20. Blue Buffer 8*.....30 seconds
21. diH2O.....30 seconds
22. 95 % Alcohol.....30 seconds
23. Eosin 515 LT.....30 seconds
24. 95 % Alcohol.....30 seconds

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UTHealth
The University of Texas
Health Science Center at Houston

School of Dentistry
Department of Diagnostic and Biomedical Sciences
Oral Pathology Laboratory

H&E Staining Procedure Using Automatic Stainer (broke down by staining jars):

25. 100 % Alcohol ..... 30 seconds
26. 100 % Alcohol ..... 30 seconds
27. 100 % Alcohol ..... 30 seconds
28. Xylene holding tank approximately ..... 2 minutes
29. Coverslip with permanent mounting medium

*** - these reagents are concentrated. Prepare working solution according to bottle directions before using. To prepare working solution: Pour two measures of concentrate into 500 ml of deionized or distilled water. One measure equals fluid filled to first line in bottle cap. Cap threads are not measure lines.**

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APPENDIX 1 - Summary of Literature Review - Lasers and Oral Biopsies

Author, Year	Title	Journal	Laser type	Type of excision	The purpose of study	Research design	Statistical analysis	Finding/Result
Ahn J H, Power S, Thickett E., *2020*	Application of the diode laser for soft-tissue surgery in orthodontics: Case series.	J Orthod. 2020 Sep 28:1465312520958706.	Diode laser	*IN VIVO* Incisions Gingivectomy Frenectomy and Incisor exposure Not cited irradiations conditions.	To demonstrate various uses of diode laser therapy in orthodontics for case reports.	Not avaiable	Not cited	Soft-tissue surgery using a dental laser is considered safe and effective, and potentially confers advantages over conventional surgery in the field of orthodontics in terms of treatment outcome and patient management.
Aldelaimi T N, Khalil, A A, *2015*.	Clinical Application of Diode Laser (980 nm) in Maxillofacial Surgical Procedures.	The Journal of Craniofacial Surgery 2015; 26:4.	Diode laser	*IN VIVO* Biopsies removed de according of the lesion format. Diode laser 980 nm 20W CW Time of exposure: range 1-2 to 6-7 min Power: 3 to 10W Fiber: not available	The aim of this study is to apply and assess the clinical usefulness of diode laser 980 nm in the treatment of different maxillofacial conditions.	**Population:** *32 patients - 22 male - 10 female Patients were examined at 3 days, 1, 2, and 4 weeks after surgery to assess pain, bleeding, edema, functions, and overall satisfaction. **Specimen Preparation:** No cited **Evaluation:** No cited **How?** No cited **Where was the measurement taken?** No cited.	No cited	12.8% - tongue-tie 16.0% - hemangioma 12.8% - pyogenic granuloma. No patient experienced pain during the surgical operation; Only 3 (9.6%) patients experienced mild pain during3 days postoperatively. The clinical application of the diode (980 nm) laser in maxillofacial surgery proved to be of beneficial effect for daily practice and considered practical, effective, easy to used, offers a safe, acceptable, and impressive alternative for conventional surgical techniques.

Allon, I. Kaplan, I. Gal, G. Chaushu, G. Allon, D M., *2014*.	The clinical characteristics of benign oral mucosal tumors.	Med Oral Patol Oral Cir Bucal. 2014 Sep 1;19 (5):e438-43.	Not cited in the text	*IN VIVO* Benign tumors of the oral mucosa.	To investigate the clinical characteristics and pre- biopsy provisional diagnoses of benign tumors of the oral mucosa.	Retrospective study **Population:** 146 benign tumors. Mean age 49.6 years Equal gender distribution. **Specimen Preparation:** Not cited **Evaluation:** Not cited **How?** Not cited **Where was the measurement taken?** Not cited	Not cited	The most prevalent tumor types were: Lipomatous (27.4%), vascular (23.3%), salivary gland (16.5%). The most frequently involved sites: Tongue, labial and buccal mucosa. The majority (98.6%) presented as non-ulcerated masses. Only 2 (1.4%) presented as ulcerated masses. Clinical provisional diagnosis: 93.3% as non malignant; 6.7% suspicion of malignancy; 42.1% benign neoplasia. This data strongly supports the need to biopsy every oral mucosal mass, since inaccurate clinical evaluation of the lesion's biological nature was a frequent event.

Alonso F C, Jornet P L, Torres M J J, Domingo A O, *2008*.	Analysis of the histological artifacts in punch biopsies of the normal oral mucosa.	Med Oral Patol Oral Cir Bucal 2008 Oct; 13(10):E636-9.	Not used laser Punch biopsy	*IN VIVO* Biopsies - were made on the Midline in the middle third of the dorsal surface of the tongue. - 8 mm diameter circular scalpel or biopsy punch.	To investigate which artifacts appear most often in punch biopsies of the healthy oral mucosa, and to determine which are attributable to the surgical technique and which are a consequence of sample processing in the laboratory.	**Population:** *186 adult male rats (Sprague-Dawley) *186 samples (dorsal lingual mucosa) **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 4 µm; - Stained with H.E **Evaluation:** - Microscopic: Magnification x100 **How?** Measurement of thermal denaturation – distance from the surgical margin to the end of visible denaturation. **Where was the measurement taken?** Two borders of lesion – not very clear.	SPSS version 12.0 (SPSS Inc, Chicago, IL, USA) statistical package for Microsoft Windows. A descriptive study was made of each variable.	Study shows use of the punch biopsies for obtaining biopsies of the healthy oral mucosa to produce few artifacts. Surgical technique: no showed splits, fragmentation or haemorrhage. Crush – showed in 31 cases. In two cases – presence of true pseudocysts. In first histological evaluation identified 23 pseudocysts.

Amaral M B F, Ávila J M S, Abreu M H G, *et al., 2015*.	Diode laser surgery versus scalpel surgery in the treatment of fibrous hyperplasia: a randomized clinical trial	Int. J. Oral Maxillofac. Surg. 2015; 44: 1383–1389.	Scalpel and Diode laser	*IN VIVO* *<u>Diode Laser:</u> λ= 808 nm Output – 2.0-2.7W Average-2.5W CW Fiber- 320 µm	To compare the effects of diode laser surgery to those of the conventional technique in patients with fibrous hyperplasia.	Randomized clinical trial **Population:** - 38 patients: 19 used scalpel and 17 used diode laser. - Limited sized lesions: flaccid to fibrous consistency, sessile or pedicle, pale to erythematous lesions; Lesions associated with dentures or parafunctional habits. **Specimen Preparation:** - 10% buffered formalin; - **Evaluation:** **How?** **Where was the measurement taken?** In their largest diameter with a millimetre rule.	Descriptive statics and association tests for comparisons between the two groups. SPSS-17.0 Software *Shapiro-Wilk: to evaluate the distribution of numerical variables (normal or non-normal) *The Mann-Whitney *U*-test: compare numerical variables *x^2 test or Fisher's exact test was applied for categorical variables. *Student's *t*-test: to compare the duration of surgery (normal distribution of the data). *Kaplan Meier method (means): time to healing (days) of the postoperative wounds. - Level of significance $P \leq 0.05$.	- Fibrous hyperplasia (76.5%) - focal fibrous hyperplasia (23.5%). - size of the fibrous hyperplasia ranged from 5 to 90mm (study group) and 8 to 60 mm (control group). No statistically significant difference in the sizes of the lesions was found when comparing the two groups. - Diode laser surgery proved to be more effective and less invasive when compared to scalpel surgery in the management of fibrous hyperplasia. - wound healing proved to be faster when using scalpel surgery.

Angiero F, Parma L, Crippa R, Benedicenti S., *2012*.	Diode laser (808 nm) applied to oral soft tissue lesions: a retrospective study to assess histopathological diagnosis and evaluate physical damage.	Lasers Med Sci (2012) 27:383-388.	Diode laser	*IN VIVO* Biopsies: Excisional - benign (< 5mm) or incisional – pre-malignant or > 5mm *<u>Diode Laser:</u> λ= 808 nm Output – 1.6-2.7W Average-2.5W CW Fiber- 320 µm	To establish if physical damage induced by the diode laser could affect the histopathological diagnosis and to evaluate the damage caused to the resection margins.	**Population:** *608 patients (269 men and 339 women; mean age 51 years). **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 4-5µm; - Stained with H.E **Evaluation:** - Microscopic 100x **How?** - Measurement of thermal denaturation – distance from the surgical margin to the end of visible denaturation **Where was the measurement taken?** - Two borders of lesion – not clear.	No statistical analysis was done Reported in %	* Diode laser is a valid therapeutic instrument for excising <u>oral lesions larger than 3 mm in diameter</u>, * induces serious thermal effects in small lesions (mean size below 3 mm). *From a clinical standpoint, it is suggested necessary that the specimens taken have in vivo a <u>diameter of at least 5 mm</u> in order to have a reliable reading of the histological sample.

Angiero F, Buccianti A, Parma L, Crippa R., *2015*.	Human papilloma virus lesions of the oral cavity: healing and relapse after treatment with 810-980 nm diode laser	Lasers Med Sci (2015) 30:747-751.	Diode Laser	*IN VIVO* Excision - benign lesion; (3 and 15 mm) *<u>Diode Laser:</u> λ= 810 nm λ= 830 nm λ= 980 nm Output – 1.6-2.8W Average – 2.2W CW Time: 2 – 3s/each time (to identify the cleavage plane). Total time for the excision – 10s. Fluence – 11-22 J/cm^2 Fiber- 300µm Irrigation with the saline solution.	To evaluate the therapeutic efficacy of Laser therapy in the treatment of oral lesions by HPV. Evaluate intervention method, recovery, VNS score (pain) and recidivism.	**Population:** *170 patients (90 men and 80 women); **Specimen Preparation:** - Fixed (without citation); - Embedded (without citation) - Sections of thickness 3 µm; - Stained with H.E **Evaluation:** - Microscopic 100x **How?** Did not measure thermal damage, but photocoagulation that can induce denaturation of viral proteins, killing the virus. **Where was the measurement taken?** Cutting margin – where the virus is located predominantly at the level of the cells of the basal layer.	Retrospective study – does not cite statistical analysis.	***Diode laser** surgery is a <u>safe, effective, and well tolerated</u> treatment for HPV – related lesions.

Arashiro D S., Rapley J W, Cobb C M, Killoy W J., *1996*.	Histologic evaluation of porcine skin incisions produced by CO2 laser, electrosurgery, and scalpel.	Int J Periodont Rest Dent 1996;16:479-491.	Scalpel Electrosurgery CO2 laser	*IN VITRO* Skin Dorsal thorax - Micro Yucatan Swine 02 swines - Eletrosurgery – 45W - Laser CO_2 – focused beam (2mm from target surface), tissue exposure 4 mm per second. Average power – not available. Fiber: not available. <u>Incisions:</u> * scalpel: 1cm length x 2 mm depth. * Laser CO_2 and Eletrosurgery: 1cm length x 1mm depth. Average power – not available Fiber: not available **Biopsies:** Incisions - Design with a marker on the dorsal thorax (8 x 5cm) - 4 subsections in this area of 2 x 5 cm - 3 incisions in each subsection	Histologically compare the healing of incisions made by CO2 laser, electrosurgery. And the scalpel.	Descriptive and Subjective analysis **Population**: 02 micro Yucatan swine **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 5-6µm; - Stained with H.E **Evaluation:** - Microscopic 25X, 100x and 400X. **How?** Measurement of zone: 1. tissue ablations (depth), 2. thermal necrosis (width) 3. extent of surface damage (width). **Where was the measurement taken?** 1. Tissue ablations= imagine line in the 90 degree angulation with relation to another horizontal imaginary line at the height of the crystal tissue, in the deepest side of the sample. 2. zone of thermal necrosis:	Not cited	The skin incisions made with the conventional scalpel produced less damage to collateral tissues and more rapid wound healing than similar Incisions made by CO_2 laser or for oral soft tissue surgery given the relatively thin epithelial and connective tissue layers overlying alveolar bone.

Araujo J G L, Araujo E M S, Rodrigues F C N, et al., *2019*	High power laser and photobiomodulation in oral surgery: Case report	J Lasers med Sci 2019; 10(1):75-78.	Diode laser	*IN VIVO* * Diode laser: Power output – 2W CW λ= 880 nm Fiber – 300 µm. E = 120 J	To show a clinical case performed at Laser Extension Project in Dentistry, Federal University of Maranhão grounded in a literature review.	**Population:** A 20-year-old female patient **Specimen Preparation:** Not applicable **Evaluation:** Not applicable **How?** Not applicable **Where was the measurement taken?** Not applicable	Not applicable	- Result was satisfactory; the technique used was simple and made it possible to perform a safe procedure and to reduce clinical time.
Asnaashari M, *2015*	Expedited removal of pyogenic granuloma by diode laser in a pediatric patient.	J Lasers Med Sci 2015;6(1):40-4	Diode laser	*IN VIVO* Lesion: - Exophytic mass, solitary sessile, red in color and soft consistency, lobulated with smooth surface which was ulcerated and covered with fibrinoleukocyter exudate on some areas. - Size: 11X13mm in diameter - Localization: right mandibular buccal gingiva, between the second primary molar and the first permanent molar. Diode laser: λ= 810 nm CW Power output = 3W Fiber – 400 µm	To show that the diode laser can be used as an alternative for the treatment of pyogenic granuloma.	**Population:** 6 year old child **Specimen Preparation:** - Fixed in a 10% buffered formalin. - Stained by Haematoxylin and eosin **Evaluation:** Not applicable **How?** Not applicable **Where was the measurement taken?** Not applicable	No statistical analysis due to the limited number of specimens.	- The use of laser for soft tissue surgeries such as removal of pyogenic granuloma would lessen stress and fear of pediatric patients and would also minimize discomfort during and after surgery.

Azevedo A S, Monteiro L S, Ferreira F, *et al., 2016*	Histological evaluation of the surgical margins made by different laser wavelengths in tongue tissues.	J Clin Exp Dent. 2016;8 (4):e388-96.	Diode lasers	*EX VIVO* **Incisions** *Er: YAG Laser (N = 40) λ= 2,940 µm non contact - power: 2W - freq: 10 Hz - short pulse: 0.2J (with air/water) - power: 2W - freq: 10 Hz - short pulse: 0.2J (without air/water) - power: 4W - freq: 10 Hz - short pulse: 0.4J (with air/water) - power: 4W - freq: 10 Hz - short pulse: 0.4J (without air/water) *CO2 laser (N = 30) λ= 10,6 µm non-contact - Power: 3,5 W (PW) Freq: 50 Hz - Power: 7 W (PW) Freq: 50 Hz - Power: 7 W (CW) *Diode Laser (N = 20) λ= 980 nm in contact - Power: 3,5 W - Power: 3,5 W boost PW. *Nd:YAG Laser (N = 10) λ= 1.06 µm Fiber: 300 µm Power: 6 W In contact Freq: 40 Hz *Electro scalpel (N = 10) Power: 5 W	To determine the macroscopic and microscopic morphological changes in the surgical margins in tongue tissue (*ex vivo*) induced by different surgical instruments, including various types of laser.	**Population:** 10 pig cadavers tongues 120 incisions - Laser beam perpendicularly to the dorse of the tongue. **Specimen Preparation:** - 240 histological preparations - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 3µm; - Stained with H.E (120 hist. preparations) and Masson Trichrome (120 hist. preparations). **Evaluation:** - Microscopic 50x and 100x **How?** Measurement of thermal – Epithelial changes in the core, loss of intraepithelial and sub epithelial adhesion, modification of connective tissue (charring and desiccation), morphology and regularity of the incision	Descriptive and inferential SPSS-22.0 Software - Kolmogorov-Smirnov: Null hypothesis - $P < 0.05$ - Sample does not follow a normal distribution in the variables under study - Nonparametric tests: *Spearman correlation test, *Mann Whitney test *Kruskal-Wallis test *Chi-Square test	***Er:YAG** laser may be the laser of choice for biopsies of the oral mucosa because of the minimum histological artefacts observed *ensuring a valid histological evaluation, followed by the CO2 laser at 3.5W in pulsed mode

				*Cold Scalpel (N = 10) Blade number 15.		**Where was the measurement taken?** Extent of Thermal Tissue Damage (microns): the greatest distance from the edge of the incision to the end of the laser thermal damage in the tissue.		
Azma E, Safavi N, *2013*	Diode laser application in soft tissue oral surgery.	J Lasers Med Sci. 2013;4(4):206-11.	Diode laser	*IN VIVO* *Diode laser case 1: λ= 810 nm (GIGGA) Power output = 3.5W CW Fiber = 200 µm. *Diode laser case 2: λ= 940 nm (Biolase) Power output = 1.5W CW Fiber = 400 µm. In sweeping motion.	To report the use of diode laser for surgical removal of exophytic tissue and pigmented tissue in two cases.	**Population:** - 53 year old woman; - 26 year old woman **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; **Evaluation:** Microscopic, but not cited the magnification. **How?** Not cited **Where was the measurement taken?**	Not applicable	- Diode lasers can be used in oral soft tissue surgery and especially small prominent lesions because of easy application, better coagulation, no need for suturing, less swelling and pain, as well as for its capability for treatment of physiologic gingival pigmentation. - Diode laser can be considered as a first choice despite periodontal surgery due to: faster action, better deepithelization, no bleeding and better repair.

Bakhtiari S, Taheri J, Sehhatpour M, *et al., 2015*	Removal of an Extra-large irritation fibroma with a combination of Diode laser and Scalpel.	J Lasers Med Sci 2015 Autumn;6(4):182-184	Diode laser Scalpel	*IN VIVO* **Biopsy Excisional** Irritation fibroma *<u>Diode laser</u> λ= 810 nm Average power – 3 W 400 µm. CW.	To report a very large irritation fibroma in right lingual side of retro molar pad with diode laser and scalpel for excision.	**Population:** patient woman, 46 years old. - Large bulky mass measuring 3.5x2.5x1 cm, pale pinkish in color, smooth surface with no ulcer and also some telangiectatic vessels, broad base, firm in consistency located in right lingual side of retromolar pad at posterior site of mouth. **Specimen Preparation:** Fixed in a 10% buffered formalin solution; **Evaluation:** - a nodular mass with dense collagen bundles with wavy and spindle shaped fibroblasts, arranged in haphazard fashion. - Covering stratified squamous epithelium demonstrated atrophy of rete ridge due to connective tissue proliferation. - Presence of focal fibrous hyperplasia.	Not cited	Laser can be considered as a good modality even for very large lesions, which are difficult to access by conventional surgery.

						- Microscopic – without citation of the magnification. **How?** The size of lesion (3.5x2.5x1 cm) **Where was the measurement taken?** Not cited		
Bargiela-Pérez P**,** *2018*	Prospective study of the 532 nm laser (KTP) versus diode laser 980 nm in the resection of hyperplastic lesions of the oral cavity.	Med Oral Patol Cir Bucal. 2018 Jan 1;23(1):e78-85.	KTP Diode laser	*IN VIVO* Biopsies on the buccal mucosa *Diode laser: λ= 980 nm Power = 1 to 10W CW Fiber = 320 µm. *KTP laser λ= 532 nm Power = 1 to 12W CW/CP Fiber = 320 µm.	To evaluate the resection of hyperplastic lesions on the buccal mucosa comparing the 532nm laser (KTP), versus diode 980nm laser, considering pain, scarring, inflammation and drug consumption that occurred postoperatively with each lasers.	Prospective **Population:** 20 patients Two groups that presents hyperplastic lesions on the buccal mucosa. **Specimen preparation:** Not applicable **Evaluation:** - VAS scale **How?** To evaluated Pain, inflammation and evolution of healing were used a VAS scale 1 to 5. **Where was the measurement taken?** Reported by the patient.	t-test chi-square Brunner Langer Mann-Whitney Fisher Test Software SPSS 20.0 (IBM, USA). - The level of significance was predetermined as 0.05.	The excision of benign hyperplastic tumors in buccal mucosa can be performed very safe and efficient with each laser, the 980 nm diode and the 532 nm KTP, used in the study. Although there is a little physical advantage in superficial absorption in the 532 nm for buccal mucosa the 980 nm works as well in clinically. After completed healing there are no differences visible or reported by patients.

Beer F, Körpert W, Passow H, *et al., 2012*	Reduction of collateral thermal impact of diode laser irradiation on soft tissue due to modified application parameters	Lasers Med Sci (2012) 27:917–921	Diode laser	*IN VITRO* Scalpel Square pieces Incision center of specimen *Diode Laser: λ= 980 nm pulsed and micropulsed mode 1)Pulsed mode: peak powers 2.5, 3.5, 4.5 W Output – 1.6-2.8W Average – 1.2, 1.8, 2,2W 2)Micropulsed mode: Peak power – 12W Average power:1.2, 1.8, 2.2W Fiber- 300µm IN CONTACT Time: 2 – 3s/each time (to identify the cleavage plane). Total time for the excision – 10s. Fluency – 11-22 J/cm^2 Fiber- 300µm	To investigate the effect of different working modes (pulsed and micropulsed) and power settings of a standardized 980 nm diode laser on collateral thermal soft-tissue damage.	**Population:** 108 bovine samples. Incisions: 24mm in length made in 24, 48 and 72 s. **Specimen Preparation:** - Fixed in a 4% neutral-buffered formaldehyde; - Embedded in paraffin block: without citation - 108 wax blocks - Sections of thickness 3 µm (rotary microtome). - Stained: Martius scarlet blue. **Evaluation:** - Histomorpho metric analysis= software (Defines Developer XP) = drew the lines between the areas in accordance with their different coloration - Microscopic – without citation of the magnification. **How?** measurement of damage thermal = different coloration **Where was the measurement taken?** incision width and depth	It only cites having performed a covariance analysis	*average power and incision depth and width, area and depth of carbonization and necrosis = without correlations *area and depth of reversible damage = correlated with average power *Pulsed mode = associated with a larger zone of carbonization, area and depth of necrosis and a greater incision width. *micro pulsed mode—maximum energy with the largest pause-to-pulse ratio: can minimize the collateral thermal damage in soft tissue. *Reduction in collateral thermal damage during the application of micro pulsed mode is not associated with a reduction in the cutting ability of the diode laser.

Beer F, Körpert W, Buchmair A G, *et al., 2013*	The influence of water/air cooling on collateral tissue damage using a diode laser with an innovative pulse design (micropulsed mode) — an in vitro study	Lasers Med Sci (2013) 28:965–971	Diode laser	*IN VITRO* Square tissue lobules - 4×4.5 cm and about 0.8 cm thick <u>Diode Laser:</u> λ= 980 nm micropulsed mode – 3W peak powers =12 W Average – 1.5 W. In contact Fiber- 300µm	To investigate the effect of water/air cooling on the collateral thermal soft tissue damage of 980-nm diode laser incisions.	**Population:** 4 pieces of capsular pork liver 3 squares pieces each liver = 36 samples. 3 Incisions: 24mm in length with a distance of 10 mm from each other were made in 72 s. **Specimen Preparation:** - Fixed in a 4% neutral-buffered formaldehyde; - Embedded in paraffin block - 36 wax blocks - Sections of thickness 3 µm (rotary microtome). - Stained: Martius scarlet blue. (protocol) **Evaluation:** -Histomorpho metric analysis = software (Definiens Developer XP) = drew the lines between the areas in accordance with their different coloration - Microscopic – by software. **How?** measurement of damage thermal = different coloration “Tissue damage” = whole area of	mean value, standard deviation 95 % confidence interval data evaluations: calculation of absolute and relative frequency. Comparisons between the groups: “test t” (water or no water) multivariate analysis of variance (water/air cooling with the three different ratios) linear contrast: calculated between the different groups— “Schaffer-test.”	*water/air cooling has an effect on the cutting efficiency of a diode laser without increasing the collateral tissue damage. *Further studies will have to clarify if improving the water/air cooling system will further minimize the tissue damage while enhancing the cutting efficiency

						tissue alteration or just the thickness of layers beneath the cut. **Where was the measurement taken?** incision width and depth.		
Belikov A V, Skrypnik A V, Shatilova K V., *2015*	Comparison of diode laser in soft tissue surgery using continuous wave and pulsed modes in vitro.	Front. Optoelectron. 2015, 8(2): 212–219	Diode laser	*IN VITRO* *Diode laser λ= 980 nm Average power – 10W. Maximum pulsed power – 20W. CW CP Fiber – 320 µm.	To compare of diode laser in soft tissue using continuous wave and pulsed modes.	**Population:** Chicken meat **Specimen Preparation:** - Fixed in a 4% neutral-buffered formaldehyde; - Embedded in paraffin block - 36 wax blocks - Sections of thickness 3 µm (rotary microtome). - Stained: Martius scarlet blue. (protocol) **Evaluation:** The crater depth and width of collateral damage in soft tissues were measured after laser action. **How?** Not cited. **Where was the measurement taken?** In the each incision.	- Stat Graphics plus 2.1	- when the laser mode was changed from CW mode to pulsed mode during laser surgery, an increase in crater depths and areas of collateral damage width were observed in the tissue. - In the CW mode: the tissue coagulation began later and carbonization and cutting occurred earlier. - The use of the hot tip instead of the clear tip increased the crater depth and reduced the width of collateral damage formed in the soft tissue.

Borchers, R., *2008*	Comparison of diode lasers in soft- tissue surgery using CW and superpulsed mode: an in vivo study.	RWTH Aachen University: Master thesis for Master of Science in Laser in Dentistry. 2008:25-55.	Diode laser	*IN VIVO* Diode laser Vision MDL-10 λ= 980 nm P_{peak} = 2,5W CW mode or 20 Hz mode Fiber: 400 µm Elexxion Claros λ= 810 nm P_{peak} = 10 mW – 50 W Pulse duration = 2,5 µs-CW Fiber: 200 µm, 300 µm, 400 µm and 600 µm. Digitally superpulsed (8 – 20.000 Hz).	To investigate in vivo, if superpulsed mode of operation can realize an improvement for surgeon and patient in soft tissue surgery.	**Population:** 26 patients (12 patients were treated by superpulsed and 14 patients were treated by CW mode). **Specimen Preparation:** Not cited **Evaluation:** Descriptive analysis. **How?** Not cited **Where was the measurement taken?** Not cited	Not cited	- The superpulsed group showed less carbonization, the cutting speed and the cut itself more defined and deeper in the superpulsed mode, and had a shorter healing time than CW-mode treatment. Conclusion: - carbonization and thermal damage of the tissues are reduced to a minimum, healing is faster; - Cutting soft tissue is faster and more accurate. - Patients have less pain and less swelling; The need for medication is reduced;
Bornstein M M, Winzap-Kalin C, Cochran D L, *et al., 2005*	The CO_2 laser for excisional biopsies of oral lesions: a case series.	Int J Periodontics Restorative Dent. 2005 Jun;25(3):221-9.	CO_2 Laser Scalpel	*IN VIVO* CO_2 laser: λ= 10.6 µm Non contact Power = 3 to 9 W CW (continuous wave) SP (Superpulsed)	To evaluate the spectrum of indications, intra- and postoperative complications, intra- and postoperative pain control, and diagnostic efficacy when using the CO_2 laser for the treatment of intraoral pathologic lesions.	Prospective **Population:** 139 patients with a total of 164 stomatologic lesions **Specimen Preparation:** **Evaluation:** Descriptive analysis **How?** Not cited	Not cited	- The 164 lesions: fibrous hyperplasia (53 lesions), maxillary labial frenu- lum (20), oral leukoplakia (13), oral squamous papilloma (12), mucus extravasation cyst (12), benign fibroma (9), ankyloglossia/tong ue tie (8), and hemangioma (7). - - incisional or excisional biopsies

						Where was the measurement taken? Not cited		cause no diagnostic problems because of collateral thermal damage of the specimen from the laser.
Capodiferro S, Maiorano E, Loiudice A M, *et al., 2008*	Oral laser surgical pathology: a preliminary study on the clinical advantages of diode laser and on the histopathological features of specimens evaluated by conventional and confocal laser scanning microscopy.	Minerva Stomatol . Jan-Feb 2008;57(1-2):1-6, 6-7.	Diode Laser	*IN VIVO* Diode laser: λ= 880 nm P = 2 – 7 W in CW Fiber = 300 µm	to verify the clinical benefits such as reduction of postoperative pain, inflammatory response and bleeding and also the benefits concerning wound healing and histopathological alterations of specimens related to diode laser surgery.	**Population:** 25 patients **Specimen Preparation:** - Fixed in 10% buffered formalin - Stain with hematoxylin and eosin. **Evaluation:** - Conventional microscopy and confocal laser scanning microscopy - To detect possible changes in the specimen related to the use of the diode laser, **How?** Not cited **Where was the measurement taken?** In the central and peripheral areas of the specimen	Not cited	The diode laser can also be used for malignant lesions, since an important factor for obtaining specimens without alterations is the choice of the laser scenario, strictly related to the clinical experience, clinical situation, location and biological nature of the lesion.

Carew J F, Ward R F, LaBruna A, *et al., 1998*	Effects of Scalpel, Electrocautery, and C02 and KTP Lasers on Wound Healing in Rat Tongues	Laryngoscope. 1998, 108(3):373-80.	Scalpel Electrocautery CO_2 laser KTP laser	*IN VIVO* **Rat tongue incision** *CO_2 laser Hollow-tube delivery system with 4 mm Spot size – 6W CW *KTP laser 4mm flexible quartz endostat Fiber – 2W CW *Scalpel *Electrocautery Cutting mode – 3W.	To evaluate wound healing of incisions created by the scalpel, electrocautery, $C0_2$ laser, and KTP laser in the, upper aerodigestive tract in animals.	**Population:** *40 wistar albino rats 4 groups of 10 rats. Scalpel, electrocautery, CO2 and KTP laser - 1 cm longitudinal incision Incisions time – 30 s. **Specimen Preparation:** - Embedded in a 10% polyvinyl alcohol; - sectioned in a plane perpendicular to the incision, into 5- to 7-µm slices; - Stained with H.E **Evaluation:** - Microscopic 40x **How?** - the measure the depth of wound lesions = reticle eyepiece. - depth of the lesion half the total distance through the wound. **Where was the measurement taken?** Reepithelialization of the wound surface, Incisional space with presence of granulation tissue (fibroblasts,	Student's t-test - comparing the experimental groups Significance level - 95% ($a < 0.05$).	*Wounds created by the scalpel – less postoperative weight loss (less pain), greater *wound depth, lower tensile strength compared to other groups. *Wounds created by KTP laser - greater weight loss (more pain), greater wound depth and lower tensile strength - poor performance by having a high degree of tissue penetration. *Wounds created by CO2 laser - greater weight loss (more pain), greater wound depth. *Wounds created by electrocautery - greater weight loss, greater depth of wound.

						neovascularization and extracellular matrix).		
Cercadillo- Ibarguren I, España-Tost A, Arnabat-Domínguez J, *et al., 2010.*	Histologic evaluation of thermal damage produced on soft tissues by CO_2, Er,Cr:YSGG and Diode lasers.	Med Oral Patol Oral Cir Bucal. 2010 Nov 1; 15 (6):e912-8.	CO_2 Er,Cr:YSGG Diode laser	*IN VITRO* ***** Scapel Square pieces Incision center of specimen *Er,Cr:YSGG λ = 2780 nm CP P= 0 – 6 W H2O+ F = 20 Hz air Diam. = 600 µm Non-contact 2mm - 1 W with 7% h2o e 11% air - 1 W without h2o e air - 2W with 7% h2o e 11% air - 2 W without air - 4 W with 7% h2o e 11% air *****CO2 λ = 10.600 nm P = 20 W (CP or CW) Non-contact 120 mm - 1w - 2w - 10 W CW - 20 W - 20 W – CP – PW 100 ms and intervals 200 ms *Diode Laser λ = 830 nm -Semiconductor (As-Ga-Al) CP and CW Diam. = 600 µm - CW = 1 – 10 W	*To perform histological evaluation of the thermal effect produced on soft tissue irradiated with CO2, Er,Cr:YSGG or diode lasers	**Population:** Porcine oral mucosa samples 240 histological preparations (120 H.E + 120 T.M) **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 3-5µm; - Stained with Hematoxylin-Eosin and Masson Trichrome stains. **Evaluation:** - Microscopic 40x **How?** - width of damaged tissue adjacent to the incision, stained positively for hyalinized tissue **Where was the measurement taken?** - Epithelial changes (Cytoplasmic and membrane modifications, loss of intraepithelial and subepithelial adhesion - Modifications of Connective tissue (charring and desiccation)	*SPSS-22.0 software *Kolmogorov-Smirnov normality test (null hypothesis) - non-parametric test: *Spearman correlation test *Mann-Whitney test *Kruskal- Wallis test *Chi-square test * The mean level chosen for all statistical tests was p = 0.0	1^{o} Diode Showed greater thermal damage among all lasers; - More cellular artifacts such as cellular hyperchromatism, intracellular vacuolization, 75% of the irradiated perimeter 2^{o} CO2 3^{o} Er,Cr:YSGG * The result of this temperature increase can be deduced by the denaturation and hyalinization of the tissue next to the irradiated area.

				- CP – pulse width: 50 µs; with intervals 100 µs; P = 2W, 5W, 10W (30% Duty Cycle)		- Morphology and regularity of the inicision – scale 0 to 4 – regular ≥ 2 and irregular ≤ 2; level 4 = high quality and 0 = worst quality. - Damage thermal (µm) - the longest distance from the incision edge to the end of the thermal damage.		

D'Arcangelo C, Di Maio F, Prosperi G D, *et al., 2007*	A preliminary study of healing of diode laser versus scalpel incisions in rat oral tissue: a comparison of clinical, histological and immunohistochemical results.	Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:764-73	Diode laser Scalpel	*EX VIVO* *Diode Laser: λ = 808 nm P – 4W and 6W. Fiber = 400 µm.	to compare wound healing of rat oral tissues after surgical procedure with diode laser or scalpel. Healing was evaluated histologically, immunohistochemically, and by measurement of 2 nitric oxide synthase isoforms (eNOS and iNOS) as intracellular messenger molecules with important immune functions. The instruments were also evaluated for performance and ease of use.	**Population:** 12 Wistar rats **Specimen Preparation:** - 7 um sections; - Stained with Hematoxylin-eosin **Evaluation:** Light microscopy Immunohistochemical **How?** **Where was the measurement taken?**	- mean and SD and - ANOVA test - $p < 0.05$	Scalpel repair = considered equivalent or better than laser repair. Histological analysis = laser incision repair was related to the beam parameters and characteristics. The 6 W power diode laser showed the worst tissue repair results, especially after 7 days. the extent of lateral epithelial damage to the edge of the wound and the extent of collagen denaturation were almost equal to the scalpel incision and laser irradiation at 4 W after 14 days.
De Falco D, Di Venere D, Maiorano E., *2020*	An overview of diode laser-assisted oral surgery	De Falco D, Di Venere D, Maiorano E (July 20, 2020) An Overview of Diode Laser-Assisted Oral Surgery. Cureus 12(7): e9297. DOI 10.7759/cureus.9297	Diode laser	*IN VIVO* Case 1: *Diode Laser: λ = 980 nm P – 1W CW Fiber = 320 µm. Case 2: *Diode Laser: λ = 980 nm P – 1W CW Fiber = 320 µm. Case 3: *Diode Laser: λ = 980 nm P – 1,5W CW Fiber = 320 µm.	To report a case series to highlight the capabilities of Diode Laser in minor and major oral surgery.	**Population:** Case 1: 45 year-old woman Case 2: 47 year-old man Case 3: 58 year-old man Case 4: 64 year-old man Case 5: **Specimen Preparation:** Not cited **Evaluation:** Not cited **How?**		Case 1: mucocele associated with leukoplakia with no sign of dysplasia. Case 2: viral papilloma. Case 3: Frictional keratosis. Case 4: venous malformation. Case 5: Squamous cell carcinoma due to the malignant transformation of proliferative verrucous leukoplakia.

				Case 4: <u>*Diode Laser:</u> λ = 910 nm P – 5W CP Fiber = 400 µm. Case 5: <u>*Diode Laser:</u> λ = 980 nm P – 2W CW Fiber = 400 µm.		Not cited **Where was the measurement taken?** Not cited		- The diode laser has general benefits of surgery in the treatment of benign, premalignant and malignant lesions of the oral cavity, as well as venous malformations.
Diamanti N, Duxbury A J, Ariyaratnam S, *et al., 2002*	Attitudes to biopsy procedures in general dental practice	British Dental Journal 2002; 192: 588–592	Not used laser Postal questionaries	Postal questionaries from 98 oral and maxillofacial surgeons; 335 general dental practioners and 220 patients attending the Oral Medicine Clinic at the University Dental Hospital Manchester.	To assess the views and attitudes of: specialists on the dental specialist surgical registers; dentists in general practice (GDPs) and patients undergoing biopsy procedures.	**Population:** 98 oral and maxillofacial surgeons; 335 general dental practioners and 220 patients **Specimen Preparation:** Not applicable **Evaluation:** Not applicable **How?** Not applicable **Where was the measurement taken?** Not applicable	Not applicable	- 70% of Specialists would discourage dental practioners undertakin biopsies (lack skills and delays in referral) - 30% considered GDPs should be able to perform simple biopsies for benign lesions. - 15% of Dentists reported they had performed oral biopsies in the last two years - 60% felt they should be competent to biopsy bening lesions. - 65% patients worried about their biopsy result - 39% would feel anxious if their

								dentist did the biopsy, although 23% were anxious when biopsied in the oral medicine clinic.
Fitzpatrick R E, Ruiz-Esparza J, Goldman M P., *1991*	The Depth of Thermal Necrosis Using the CO_2 laser: A comparison of the Superpulsed Mode and Conventional Mode	J Dermatol Surg Oncol 1991;17:340-344.	CO_2 laser	*EX VIVO* *CO_2 laser: λ = 10.6 µm CW SP Spot Size: 2,.5mm Differents pulse durations: 0.05, 0.1, 0.2, 0.5 seconds.	Not cited	**Population:** 16 individuals 32 specimens **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness; - Stained with Hematoxylin-Eosin **Evaluation:** Not cited **How?** considered the depth of coagulation necrosis, as well as certain morphological changes, such as basal cell vacuolization, spindle alteration of nuclei, and nuclear smudging **Where was the measurement taken?** Not cited	Not cited	When precise control of thermal damage with use of the CO_2 laser is necessary, the use of the superpulsemode with a second duty cycle of repeated 50 ms pulses gives the least risk of peripheral thermal damage and resultant scarring when single pulses are used. Further studies are necessary to determine whether this will result in a clinically advantageous method of using the CO_2 laser.

Fornaini C, Merigo E, Sozzi M, Rocca JP, Poli F, Selleri S, Cucinotta A.	Four Different diode lasers comparison on soft tissues surgery: a preliminary *ex vivo* study.	Laser Therapy 25.2: 105-114 (2016).	Diode laser	*EX VIVO* *<u>Diode laser</u>: λ = 808 nm λ = 980 nm λ = 1470 nm λ = 1950 nm P = 2W and 4W CW Fiber = 320 µm.	To compare the performance of 4 different diode laser (808, 980, 1470 and 1950 nm) in oral soft tissue surgery on *ex vivo* models. * recording: - thermal increase and the evaluation - the epithelial changes, - connective tissue modifications, - presence of vascular modifications, -muscular damage, -incision morphology, - width of tissue injuries - overall width of tissue modification.	**Population:** *2 fresh beef tongues - Ventral portion: 02 samples dimension: 15 x 10 mm To perform a linear cut of 5 cm, at a speed of 5 mm/sec for each wavelength used. Thickness: 4 mm **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 5 µm; - Stained with Haematoxylin and eosin. **Evaluation:** - histopathological setions were evaluated under low and high power light microscopy. - Evaluated by two pathologists - Magnifications: 40X and 100X for measurement of tissue injuries widths. - The extension of tissue injuries was measured with an ocular micrometer. - The area with the most evident damage, perpendicular to the cut margin,	ANOVA	- Mean time necessary to perform) the excision varied 112 seconds and 271 seconds. - The most significant peal temperature was shown for 808 nm at 4W (41.2 ºC) and the lowest for 1950 nm at 2W (34.9 ºC). - The quality of incision was better and the width of overal tissue injuries was minor in the specimens irradiated at higher wavelength (1950 nm) at lower power (2W) due to its high absorption into the water.

						was chosen for the evaluation. **How?** **Where was the measurement taken?** The border of the mandible		
Goharkhay K, Moritz A, Wilder-Smith P, Schoop U, Kluger W, Jakolitsch S, Sperr W.	Effects on Oral Soft Tissue Produced by a Diode Laser In Vitro	Lasers in Surgery and Medicine 25:401-406 (1999).	Diode laser	*IN VITRO* INCISIONS IN PIG MANDIBLES *<u>Diode Laser</u> λ = 810 nm Power = 0,5 – 1,5W Pulse = 2 – 32 ms Fiber – 200 – 400 µm CW CP 25 Hz Short pulse: 30 ms 50 Hz Short Pulse: 10 ms	To determine incision characteristics and soft-tissue damage resulting from standardized incisions using a wide range of laser modes and parameters of a diode laser at 810 nm.	**Population:** *17 fresh pig mandibles - 06 standardized incisions per laser parameter combination - 3cm in length were made in the oral mucosa parallel to the border of the mandible. - 3 incisions per parameter were positioned 5 mm below the gingival margin - 3 in the thicker soft tissue 5 mm from the lower border of the mandible. **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; 198 wax blocks - Sections of thickness 6 µm; - Stained with Serius Red	Do not describe	*The remarkable cutting ability and the tolerable damage zone clearly show that the diode laser is a very effective and, because of its excellent coagulation ability, useful alternative in soft-tissue surgery of the oral cavity.

						Evaluation: - no Citation. **How?** Measurement were made from 15 slides per parameter and incision site. **Where was the measurement taken?** The border of the mandible.		
Gold S I, Vilardi M., *1994*	Pulsed laser beam effects on gingiva.	J Clin Periodontol 1994, 21(6):391-6.	Nd:YAG	*IN VIVO* 24 specimens of gingival tissue *<u>Nd:YAG</u> λ = 1064 nm Power = 1,25W and 1,75W Frequency = 20Hz Treatment time: 2 to 3 min. Pulse = 2 – 32 ms Fiber = 320 µm	To evaluated the efficacy of a low-power pulsed laser in removing pocket lining epithelium in humans with moderate periodontitis.	**Population:** 6 patients **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; **Evaluation:** Light microscope at 25 and 100 × magnification. **How?** Not cited **Where was the measurement taken?** measurement of the periodontal pocket		- 83% of the cuts showed complete removal of the epithelium. - 17% had remnants of basal cell lines at the sulcular margin. - No necrosis or carbonization in the underlying connective tissue. - The pulsed Nd-YAG laser can remove the epithelium from the lining of the moderately deep periodontal pockets.

Gontijo I, Navarro R S, Haypek P, *et al., 2005*	The applications of Diode and Er:YAG lasers in labial frenectomy in infant patients.	J Dent Child. 2005; **72**: 10 -15	Diode laser Er:YAG	*IN VIVO* Frenectomy *<u>Diode laser</u> λ = 810 nm Power = 1.5W Fiber = 6000 µm CW in contact *<u>Er:YAG</u> λ = 2,940 nm Power = W Pulse repetition = 10 – 20 pps Pulse width = 200 – 500 µm CP Non contact (2 mm to target tissue)		**Population:** 24 month-old female **Specimen Preparation:** Not applicable **Evaluation:** Not applicable **How?** Not applicable **Where was the measurement taken?** Not applicable	Not cited	- It is necessary for the professional to understand the characteristics of the laser as well as its action inside the tissues, as this technology is a good ally to the treatment of pediatric patients.
Hanby D F, Gremillion G, Zieske A W, *et al., 2011*	Harmonic Scalpel versus flexible CO2 laser for tongue resection: A histopathological analysis of thermal damage in human cadavers.	World Journal of Surgical Oncology 2011, 9:83	CO_2 laser Scalpel	*IN VITRO* Incisions similar to tongue tumor resection *<u>Harmonic Scalpel</u> Power: 5W *<u>CO2 laser</u> Power: 13W, 16W and 18W.	To compare the tissue effects of the harmonic scalpel and PBFA carbon dioxide laser in tongue resections using a human cadaveric model.	**Population**: *02 fresh human cadaver heads - 03 cadaveric tongues samples were analyzed – thermal damage with harmonic scalpel - 05 cadaveric tongue samples were analyzed for thermal damage with CO_2 **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections: Non citation - Stained: H&E **Evaluation:** - Magnification 10x	Statistical Package T – test = to compare thermal depth between harmonic scalpel and CO_2 laser	*The CO_2 fiber causes less depth of thermal damage when compared with harmonic scalpel

						How? **Where was the measurement taken?** The tongue was exposed and the incisions were made in it.		
Janda P, Sroka R, Mundweil B, Betz C S, Baumgartner R, Leunig A.	Comparison of thermal tissue Effects Induced by contact application of fiber guided laser systems	Lasers in Surgery and Medicine 33:39 – 101 (2003)	Ho:YAG Nd:YAG Diode-laser	*IN VITRO* Incisions on muscle tissue of the turkey *Diode Laser λ = 830 nm CW *Diode Laser λ = 940 nm CW *Nd:YAG laser λ = 1,064 nm CW *Ho:YAG laser λ = 2,080 nm Free – running pulsed Freq: 3-20 Hz Pulse duration: 250 µs Fiber Diameter: 400 µm - three irradiations for each laser type power settings of 5, 10, 15, and 20 W Ho:YAGlaser: 1 J at repetition rates of 5, 10, 15, and 20 Hz.	To compare the effects caused by the interaction between light and tissue when using fiber guided laser systems in contact mode with respect to ablation, carbonization, and coagulation.	**Population:** - muscle tissue of the turkey. - specimens were cut into flat pieces (thickness: = 1 cm) - three irradiations for each laser type was performed (5, 10, 15, and 20 W) and Ho:YAG laser with 5, 10, 15 e 20 Hz. **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 4-5µm; - Stained with H.E **Evaluation:** - Microscopic 16x **How?** carbonization effects on the carbonization surface were subjectively graded according to the induce color of there sulting	*The non-parametric Friedmann's test for related variables. *The measured values were regarded to be significantly different, if the Friedmann's test revealed P≤ 0.001. *values and the standard deviations for the measurements (n=9) were displayed in diagrams.	*the mode of laser application (contact or non-contact) considerably influences the induced thermal tissue effects. *Ho:YAG-laser treatment in contact application caused considerably deep and wide ablation craters into the tissue * Ho:YAG laser treatment in contact application holds the risk of injury to deeper structures, * Nd:YAG laser application in contact mode revealed broad carbonization zones at the tissue surface with little overall ablation capabilities low coagulation properties in the depth and at the surface of the

						carbonization zones. *No histological evaluations were performed **Where was the measurement taken?** Depth of the incision – not very clear		tissue in contact treatment *Nd:YAG laser showed severe carbonization at the surface with only small adjacent coagulation areas
Jin J Y, Lee S H, Yoon H J, *2010.*	A comparative study of wound healing following incision with a scalpel, diode laser or Er,Cr:YSGG laser in guinea pig oral mucosa: A histological and immunohistochemical analysis	Acta Odontologica Scandinavica, 2010; 68: 232–238	Scalpel Diode laser Er,Cr:YSGG	*IN VIVO* Incision in oral mucosa –Pig * <u>Scalpel:</u> 03 types of wound were randomly made using either a stainless-steel scalpel *<u>Diode Laser</u> 01 incision Λ = 810 nm CW Power: 2W Pulse length: 0.5 ms aiming beam = 635 nm (red spectrum) Fiber: 300 µm Non contact * <u>Er,CR:YSGG</u> 01 incision Λ = 2.78 mm Power = 3W + 15% water + 18% air spray pulse duration = 140 ms (repetition rate of 25 Hz) energy density: 300 mJ/cm2	To compare wound healing following incision witheither a scalpel, a diode laser or an Er,Cr:YSGG laser in guinea pig oral mucosa, by assessing the histology and immunohistochemistry (TNF-α and TGF-β1) of wounds.	**Population:** * 24 male guinea pigs *15 mm in length and was performed to the surface of the periosteum with no flap reflection. * distance between each incision was »15 mm **Specimen Preparation:** - Fixed in a 10% neutral buffered formalin; - Embedded in paraffin block; - Sections of thickness 4 µm; - Stained with H.E **Evaluation:** - Microscopic 100x **How?** Measurement by an inflammatory response using a scale ranging from 0 to 3 based on the degree of filtration of neutrophils,	Do not describe	*the damage resulting from using a scalpel was lower when compared to that after the laser surgical procedures for early wound healing. * The cellular injury was higher for diode laser wounds than for Er,Cr:YSGG laser wounds. *the diode laser can be considered a good incisional device for oral mucosa incisions * More tissue damage resulted from its use compared to that using a scalpel or an Er,Cr: YSGG laser

				spot size: 0.6 mm non-contact mode distance to the soft tissue: 0.5 mm - 1.0 cm		histiocytes and lymphocytes. **Where was the measurement taken?** histological appearance of the wound produced.		
Kardos T B, Holt T Ferguson,	Histological evaluation of the effect of a miniature carbon dioxide laser on oral mucosa	Int. J. Oral Maxillofac. Surg. 1989; 18: 117-120.	CO_2 laser	*IN VIVO* Rectangular Lesions in sheep tongues. *<u>CO_2 lasers:</u> λ = 10600 nm length = 24 cm output = 4 W spot = 0.2 mm (diameter) focal point = 2.5 mm water-cooled	To determine the effective- ness of the infra-red beam from the miniature carbon dioxide laser at re- moving oral tissues, and to investigate the pattern of healing in response to such treatment.	**Population:** * sheep tongues Rectangular lesions, approximately 1 cm x 0.5 cm, **Specimen Preparation:** - Fixed in a 10% neutral buffered formalin; - Embedded in paraffin block; - Sections of thickness 5 µm; - Stained with H.E and phloxine. **Evaluation:** - Microscopic 50x, 67x 125x, 150x . **How?** Measurement by 100 µm deep zone in the lamina propria. **Where was the measurement taken?** On the lateral margins of sheep tongues.	Do not describe	* The miniature CO2 laser (vaporizes epithelium and superficial connective tissue) may be valuable for the treatment of some lesions on the oral mucosa. *Some of its advantages: portability, without complex ancillary clinics, good lighting, makes it potentially useful for geographic regions where oral leukoplakia is common and in a widely dispersed population.

Kende P, Gaikwad R, Yuwanati M, *et al., 2011*.	Application of Diode Laser in Oral Biopsy: Removal of White Patch Over Tongue - A case report.	Journal of Indian Dental Association. 2011;5:985-7.	Diode laser	*IN VIVO* Excisional biopsy White patch (1x0.5cm) on right posterior one third lateral border of tongue. *<u>Diode laser:</u> λ = 940 nm	To discuss the use of diode laser as a vital tool in the removal of white lesions on the tongue with histological findings.	**Population:** 42 year old male **Specimen Preparation:** Not cited **Evaluation:** Not cited **How?** Not cited **Where was the measurement taken?** Not cited	Not applicable	Benign lesion - exophytic growth covered by hyperkeratized stratified squamous epithelium with mild dysplasia. - Intraepithelial keratinization. - basal cell layer slightly hyperchromic and intact, without infiltration of connective tissue.
Kundoor V K R, Patimeedi A, Roohi S. *et al., 2015*	Efficacy of diode laser for the management potentially malignant disorders.	J Lasers Med Sci 2015 Summer;6(3):120-123	Diode laser	*IN VIVO* White lesions *<u>Diode laser:</u> λ = 980 nm P = 4W In contact CW Fiber = 400 µm	To determine the efficacy and safety of diode lasers in the management of potentially malignant lesions (oral leukoplakia [OL] and oral lichen planus [OLP]).	**Population:** 10 patients: - 05 Oral leukoplakia - 05 lichen planus **Specimen Preparation:** Not cited **Evaluation:** Not cited **How?** Not cited **Where was the measurement taken?** Not cited	Not applicable	- 30% (3) of the patients complained of moderate pain - 70% (7) patients of mild pain. - Diode lasers provide acceptable clinical improvement of potentially malignant lesions with minimal side effects.
Makki F M, Rigby M H, Bullock M, *et al., 2014.*	CO2 laser versus cold steel margin analysis following endoscopic excision of glottic cancer.	Journal of Otolaryngology - Head and Neck Surgery 2014, 43:6	CO2 laser Steel phonomicro surgical	*IN VIVO* *<u>CO2 laser:</u> λ = 10600 nm P = 2-4W In contact Ultrapulse mode Fiber = 400 µm	To compare the suitability of CO_2 laser with steel instruments for margin excision in transoral laser microsurgery.	Prospective randomized blinded study. **Population:** Patients	Stata v11.2 (StataCorp, Texas) - Fisher exact tests - t-test was used to determine the	- both steel instruments and CO2 laser cause a significant degree of artifact that can interfere with the accurate assessment of the

						Specimen Preparation: - fixed in 10% formalin - paraffin-embedded - 5-micron sections - stained with hematoxylin and eosin **Evaluation:** **How?** **Where was the measurement taken?**	relationship between harvest technique and margin size. - $p < 0.05$	margin in TLM for early glottic cancer. - The use of lasers to collect margins in our study was not associated with increased gross rates of non-interpretability for malignancy, but it was associated with a growing artifact that affects the pathologist's ability to assess dysplasia.
Manthur E, Sareen M, Dhaka P, *et al., 2015*	Diode Laser Excision of Oral Benign Lesions.	J Lasers Med Sci 2015 Summer;6(3):129-132	Diode laser	*IN VIVO* *<u>Diode laser:</u> λ = 810 nm In contact CW Fiber = 400 µm Case 1: P = 1.4W Case 2: P = 2W Case 3: P = 0.7W Case 4: P = 0.8W	To discuss the use of diode laser as a vital tool in excision of benign soft tissue lesions of oral cavity with histological findings.	**Population:** 04 patients Case 1: 50 year old man - nontender, pedunculated, fibrous oval tumor arising from the palatal mucosa measuring about 3 cm × 1.5 cm in size. Case 2: 2 nontender, pedunculated, firm and smooth tumors on lower labial mucosa with underlying oral submucous fibrosis. Case 3: nontend-er, pedunculated, exophytic papillomatous nodule with numerous blunt	Not cited	Case 1: pyogenic granuloma. Case 2: inflammatory fibrous hyperplasia Case 3: squamous papilloma Case 4: pyogenic granuloma. - Diode lasers are rapidly becoming the standard of care in contemporary dental practice and can be used for excisional biopsies of oral soft tissue lesions with minimal problems in histopathological diagnosis.

						finger like projections. Case 4: a tender, soft pedunculated lesion in relation to 41 and 42 with profuse bleeding on probing. **Specimen Preparation:** Not cited **Evaluation:** Not cited **How?** Not cited **Where was the measurement taken?** Not cited		
Merigo, E, Clini F, Fornaini C., *et al., 2013.*	Laser-assisted surgery with different wavelengths: a preliminary *ex vivo* study on thermal increase and histological evaluation	Lasers Med Sci (2013) 28:497–504	CO_2 LASER KTP Er:YAG Nd:YAG GaAlAs	*EX VIVO* **INCISIONS IN CALF TONGUES** model of soft tissue surgery procedures *CO_2 (λ = 10.600 nm) Focal Point = 20 mm Spot size = 0.4mm 3W – CW – PD= 2,388 W/cm^2 5W – CW – PD= 3,980 W/cm^2 5W – CP-PD= 3,980 W/cm^2, pulse duration= 300µs, f= 500Hz *KTP (λ = 532 nm) fiber = 320µm in contact mode: a) 2W-CW-PD= 2,500W/cm^2	To compare different laser wavelengths in relation to both thermal increase and "histological quality" in a model of soft tissue surgery procedures	**Population:** * Freshly extracted calf tongues * In the tongue margins, areas measuring about 2 cm 2 (2×1 cm) were identified and circumscribed. * thermal * 04 naked-bead chrome–alumel (K-type) thermocouples with a 0.5-mm diameter probe sensitive to temperature variations between −40°C and 250°C to evaluate thermal increase in the depth of the specimen.	ANOVA test P<0.05 (significant) P<0.01 = very significant P<0.001 = extremely significant.	* The Er:YAG laser was the device with the least influence on the thermal increase * CO2 and diode laser revealed good histological quality

				b) 4W-CW-PD= 5,000 W/cm^2 *Er:YAG (λ = 2,940 nm) VSP - 100µs, non-contact, focal point = 8mm, spot size = 0.9mm, E= 250 Mj, f= 20 Hz with air and water spray. *Nd:YAG (1,064 nm) VSP = 100µs Fiber = 320µm, in contact, P= 4W, f= 80Hz, PD = 5,000 W/cm^2 *GaAlAs (808 nm) fiber = 320µm in contact mode a. 3W – CW – PD= 3,750 W/cm^2 b. 5W – CW – PD= 6,250 W/cm^2		**Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 5µm; - Stained with H.E **Evaluation:** - Microscopic 40X and 100x **How?** tissue modifications were evaluated in every part of the tissue (bottom, middle and margins) - Epithelial changes - Connective tissue modifications - Presence or absence of vascular modifications - Incision morphology **Where was the measurement taken?** tissue modifications were evaluated in every part of the tissue (bottom, middle and margins)		

Monteiro L, Delgado M L, Garcês F., *et al., 2019*	A histological evaluation of the surgical margins from human oral fibrous-epithelial lesions excised with CO2 laser, Diode laser, Er:YAG laser, Nd:YAG laser, electrosurgical scalpel and cold scalpel.	Med Oral Patol Oral Cir Bucal. 2019 Mar 1;24 (2):e271-80.	CO_2 Diode laser Er:YAG Nd:YAG Electrosurgical scalpel Cold scalpel	*IN VIVO* *CO_2 (λ = 10.600 nm) Spot size = 0.5mm P = 4W PD = 2040.8W/cm^2 E = 50 mJ f= 80Hz *Diode laser (λ = 980 nm) fiber = 300µm in contact mode f= 50 Hz E = 70mJ P = 3.5W *Er:YAG (λ = 2,940 nm) focalized - non-contact, spot = 0.5 mm f= 20 Hz E – 200 mJ P = 4W PD = 2040.8 W/cm^2 *Nd:YAG (1064 nm) Fiber = 300µm, in contact, P= 4W, f= 40Hz, E = 100 mJ *Electrosurgical scalpel in contact mode P = 5W *Scalpel blade 15C	To determine which instrument produce highest and lower tissue damage and finally if some of these instruments could impair the normal histo- logical diagnosis of these lesions.	Retrospective **Population:** 130 patients with diagnosis of a benign epithelial, fibrous or fibrous-epithelial hyperplasia, and lesion within oral cavity. **Specimen Preparation:** - fixed in a 10% formalin solution - Sections of the 3 µm - Stained with H E. **Evaluation:** - ZEISS AxioLab A1® microscope **How?** At magnification of 40x **Where was the measurement taken?** according to the size of the lesion.	- SPSS-24.0 software - descriptive and inferential statistics - Shapiro-Wilk test (analyse the normality of the numerical variables) showed a non-parametric distrbution. - Spearman correlation test, Mann-Whitney test, Kruskal-Wallis test = to analyze possible relations between continuous variables; - Chi-Square test (categorical variables) - Differences were considered statistically significant at *P*<0.05.	- lasers can be used for the excision of benign oral fibroepithelial hyperplasia, without limiting the histological diagnosis. - The Er: YAG laser has proven to be a laser with little extension of tissue damage and with good regularity of the incision.
Munishekar M S, Reddy K M, Ahmed S A., *et al., 2011*	Conventional Scalpel vs Laser Biopsy: A comparative Pilot Study	International Journal of laser Dentistry, 2011;1(1):41-44.	Er, Cr: YSGG Diode laser	*IN VIVO* * Er, Cr: YSGG λ = 2,780 nm P = 2W *Diode laser λ = 940 nm P = 2W	To evaluated the effect of lasers specifically on the peripheral architeture of suspected oral lesions.	**Population:** 6 patients **Specimen Preparation:** - fixed in a 10% formalin solution - Sections of the 3 µm - Stained with H E.	Not cited	- Er,Cr: YSGG has been more effective by producing least tissue distortion/artefacts.

				*Diode laser λ = 940 nm P = 3W		**Evaluation:** Not cited. **How?** Not cited. **Where was the measurement taken?** Not cited.		
Palaia G, Del Vecchio A, Impellizzeri A., *et al., 2014*	The Scientific World Journal Volume 2014, article ID 345685, 6 pages	Histological *Ex Vivo* Evaluation of Peri-Incisional Thermal Effect Created by a New-Generation CO2 Superpulsed Laser	CO_2 laser	*EX VIVO* Biopsies in pig tongues *CO_2: λ= 10.600 nm (ultra-sped laser: SmartUS20D, DEKA, Florence, Italy) Power= 2 to 4W CW Pulsed wave (PW= 50Hz	The evaluation of the histological effects of a new-generation superpulsed CO2 laser through an“ex vivo” study	**Population:** *30 samples from pig cadaver tongues. *depth of approximately 0,5 cm and a width of approximately 1 cm. *6 groups (from A to F) and each group consisted of 5 samples. * Control group: scalpel **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness: No citation - Stained with H.E **Evaluation:** - Microscopic 40x **How?** The width of thermal damage in the peri-incisional epithelial and connective tissue was measured.	Graphpad Prism 5 software showed no significant differences among the groups. Test de Dunn (comparative between the groups).	***CO2** – epithelial damages, **CW** – 22 – 1620 µm *Connective tissue: 124 – 347 µm CP – epithelial damages: 17 - 111µm Connective tissue: 99 – 398 µm. No difference!!

						Where was the measurement taken? borders of lesion – not very clear		
Pié-Sánchez J, España-Tost A J, Arnabat-Domínguez J., *et al., 2012*	Comparative study of upper lip frenectomy with the CO_2 laser versus the Er,Cr: YSGG laser.	Med Oral Patol Oral Cir Bucal. 2012 Mar 1;17 (2):e228-32.	CO_2 laser Er,Cr: YSGG laser.	*IN VIVO* Frenectomy *CO_2: λ= 10.600 nm focused CW P = 5W Spot diameter = 0.8 mm PD – 1000 W/cm^2 *Er,Cr: YSGG λ=2780 nm Pulse duration = 140 and 200 µs f = 20 Hz P = 1.5 W (12% water, 8% air) Spot = 0.6 mm Fiber = 600 µm	To compare upper lip frenulum reinsertion, bleeding, surgical time and surgical wound healing in frenectomies performed with the CO_2 laser versus the Er, Cr:YSGG laser.	**Population:** 50 randomized pediatric patients **Specimen Preparation:** Not cited **Evaluation:** Not cited **How?** Not cited **Where was the measurement taken?** the vertical axis of the frenulum until the wound presented a linear shape	Not cited	- the use of either type of laser is useful for a simple frenectomy due to their numerous advantages.
Pogrel M A, McCraken K J, Daniels T E. *et al., 1990*	Histologic evaluation of the width of soft tissue necrosis adjacent to carbon dioxide laser incisions	Oral Surg Oral Med Oral Pathol, 1990;70:564-8.	CO_2 laser	*IN VIVO* Intra oral excisional biopsies * **CO2 laser** (Xanar Articulator -Coherent Inc., Palo Alto, Calif.) *Ø = 1 mm (focused lens) at P=17.5 W (2320 W/cm2). Pulsed Mode	The objective of this study is to objectively measure the width of necrosis of soft tissues for the CO_2 laser incision in 5 different soft tissue types: epithelium, muscle, connective tissue (dense bundles of collagenous fiber and loose fibroadipose tissue), and salivary gland epithelium.	**Population:** *23 specimens intraoral soft tissue lesions **Specimen Preparation:** - Fixed in a 10% - buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 6 µm; - Stained with H.E **Evaluation:** - Microscopic 100x	* The mean and standard deviation of tissue necrosis in microns *analysis of variance *Newman-Keuls correction. * The mean level chosen for all statistical tests was p = 0.05	*The widest zone of necrosis was seen in dense fibrous tissue, mucosal epithelium, and muscle. *Significantly less necrosis was seen in loose connective tissue and salivary glands. * The range of thermal necrosis in different tissue types is probably based on the water content within

						How? Measurements the section length of each tissue type present within the specimen. Necrosis - measuring the depth of microscopic changes, including (Loss of intercellular boundaries, Intracellular vacuolations, loss of intracellular detail and changes in staining reaction. **Where was the measurement taken?** the limits of the clinical margins of the lesion.		each type. A cellular partially homogenized zone of reversible thermal damage up to 500 µm in width was visible adjacent to the zone of thermal necrosis.
Rizoiu I M, Eversole L R, Kimmel A I, *et al., 1996*	Effects of an erbium, chromium: Yttium, scandium, gallium, garnet laser on mucocutanous soft tissue.	Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82:386-95.	Er,Cr:YSGG laser	*IN VIVO* Incisions in the ventral tongue, ear skin and dorsal skin	To evaluates the Er,Cr:YSGG effects of laser on soft tissue cutting and subsequent wound healing.	**Population:** 30 rabbits **Specimen preparation:** - Gluteraldehyde 4%; - fixed in osmium tetroxide, cleared - embedded in SPURR (Spurr's media) - Stained with uranyl acetate and lead citrate **Evaluation:** Siemens transmission electron	Not cited	- Er,Cr:YSGG laser system induces mucosal and cutaneous wounds that heal comparable with scalpel and punch biopsy wounds. - histopathological artifactual changes induced by this laser are insignificant; - Er,Cr:YSGG may be of use for diagnostic as well as therapeutic applications.

						microscope **How?** Magnification of x13 **Where was the measurement taken?** In the linear incisions in ventral tongue, ear skin and dorsal skin.		
Romanos G E, Nentwig G H., *1999*	Diode laser (980 nm) in oral and maxillofacial surgical procedures: clinical observations based on clinical applications.	Journal of Clinical Laser Medicine & Surgery.	Diode laser	*IN VIVO* *<u>Diode laser</u> λ= 980 nm P= 15W fiber – 200-400 µm. CW CP with pulse duration = 0.1-99.9 sec Case 1: P = 5W CW Case 2: P = 6-10W noncontact Case 3: P = 8-10 W CW	To examine the wound healing of soft tissue after the application of a diode laser (980 nm) in oral surgical procedures.	**Population:** 22 pacients **Specimen Preparation:** Not cited **Evaluation:** Not cited **How?** **Where was the measurement taken?** Not cited	Not cited	Case 1: Fibroma Case 2: Hemangioma Case 3: Adenoma sublingual salivary gland * The clinical application of the new diode (980 nm) laser in oral and maxillofacial surgical proce-dures seems to be of beneficial effect for daily practice.
Romanos G E, Henze M, Banihashemi S, *et al., 2004*	Removal of epithelium in periodontal pockets following diode (980 nm) laser application in the animal model: an in vitro study.	Photomed Laser Surg. 2004 Jun;22(3):177-83.	Diode laser	*IN VITRO* *Diode laser λ= 980 nm P= 2W and 4W fiber – 300 µm. Time – 15 sec CW	to examine the removal of the epithelium in the periodontal pocket using a diode (980 nm) laser in comparison with the conventional techniques in an	**Population:** 10 pig buccal pockets **Specimen Preparation:** - Fixed in a 10% - buffered formalin solution;	Not cited	- No epithelial remmants were found. - The low-power laser removes the epithelium from the thin pounch regardless of the

					animal experimental model.	- Embedded in paraffin block; - Sections of thickness 6 µm; - Stained with H.E **Evaluation:** Not cited **How?** **Not cited** **Where was the measurement taken?** Not cited		examiner's level of surgical experience. - The high power configuration showed significante damage to the underlying connectivie tissues. - The diode laser (980nm) used in periodontal tissues led to complete epithelial removal compared to conventional methods of treatment with hand instruments.
Romeo U, Libotte F, Palaia G, Del Vecchio., *et al., 2012*	Histological in vitro evaluation of the effects of Er:YAG laser on oral soft tissues	Lasers Med Sci (2012) 27:749–753	Er:YAG laser	*EX VIVO* Incisions in the tongues of swine *<u>Er: YAG laser</u> λ 2,940 nm E= 60 mJ /150 mJ, F= 21 to 53 J/cm2 fiber - 600 µm. 1) E= 60mJ Freq = 30 Hz Fluence = 21 J/cm^2 Power = 1.8 2) E= 80 mJ Freq = 30 Hz Fluence = 28 J/cm^2 Power = 2.4 W 3) E= 100 mJ Freq = 30 Hz Fluence = 35 J/cm^2	To evaluate the histological effects of the Er:YAG laser with gradually increasing settings for power, fluence and irradiance, without water cooling in tissue samples obtained during biopsy procedures.	**Population:** * five cadaver swine tongues (n=9) *five groups (groups 1-5) *score of 0 to 3 for each sample indicating the degree of peripheral thermal damage **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 3 µm; - Stained with H.E	Qualitative method was chosen because of the lack of micrometric software.	*The Er:YAG laser produced less damage *Can be safely used in oral biopsy investigations while ensuring a successful histological evaluation,

				Power = 3 W 4) E = 130 mJ Freq = 30 Hz Fluence = 46 J/cm^2 Power = 3.9 W 5) E= 150 mJ Freq = 30 Hz Fluence= 53 J/cm^2 Power = 4.5 W *<u>Scalpel</u>		**Evaluation:** - Microscopic 10X magnification with micrometric lens and application the conversion factor for the enlargement (0.035 mm / 70 pins, corresponding to 5 μm) **How?** Measurement of thermal damage - cellular columns damaged. **Where was the measurement taken?** borders of lesion – not very clear		

Romeo U, Russo C, Palaia G,. *et al., 2014*	Biopsy of different Oral Soft Tissues Lesions by KTP and Diode Laser: Histological Evaluation.	The Scientific World Journal vol. 2014, article ID 761704, 6 PAGES	Diode laser KTP laser	*IN VIVO* Excisional biopsies in oral lesions. *<u>Diode Laser</u> λ= 808 nm (SOL, Den Mat Italia, Italy) - power: 2W in CW, - fluence: 2400J/cm2, - fiberspot: 320μm; *<u>KTP Lasers</u> λ= 532 nm (SmartLite, DEKA, Italy) - power: 1.5W in PW, - fluence: 212J/cm2, - - fiber spot: 300μm.	To analyze the tissue fragments removed by laser surgery, to assess the epithelial and connective tissue damage caused by its thermal effects	**Population:** *17 patients = 17 oral benign lesion size - 0,5 and 1cm of diameter **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections (conventional); - Stained with H.E (Conventional) Evaluation: - Microscopic 5X and 10x How? - Quantitative evaluation carried out a measurement in millimeters. - Epithelial and connective tissue in thermal alterations. - connective and epithelial damage have been evaluated in terms of charring and coarctation. **Where was the measurement taken?** Peripheral damage (considering that the morphological and structural characteristics of the various lesions could strongly influence the tissues response to the laser action).	calculating the arithmetic mean and standard deviation of the different values.	*The perilesional damage did not compromise the morphological and structural characteristics of the specimens.

Suter V G A, Altermatt H J, Mettraux G, *et al., 2010*	CO_2 and Diode laser for excisional biopsies of oral mucosal lesions: a pilot study evaluating clinical and histopathological parameters	Schweiz Monatsschr Zahnmed Vol. 120 8/2010	CO_2 laser Diode laser	*IN VIVO* Excisional biopsies in oral lesions *<u>CO2 laser</u> λ = 10,6 µm Group A: Ø = 0,2mm, CW, Power = 5 W. Group B: Ø = 0,2mm, CP Power = 4,62W Freq: 140 Hz Pulse width = 400 µs, E= 33 mJ. *<u>Diode laser</u> λ = 810 nm (CP) Group C fiber = 400 µM Power = 5.12 W Pulse width = 10 µs Freq = 20.000 pulses per second P = 25W/pulse.	* To evaluate the histopathological characteristics and suitability of CO2 and diode lasers for performing excisional biopsies of similar lesions of the oral mucosa. *The thermal damage zone of the three lasers and intraoperative and postoperative complications were assessed and compared.	**Population**: *15 patients **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 4-5µm; - Stained with H.E **Evaluation:** - Microscopic 100x **How?** Measurement the maximal width of the collateral damage zone (µm) **Where was the measurement taken?** The border of lesion – not very clear.	* Fisher's exact test (comparison of the categorical variables); * Kruskal Wallis (continuous variables) * 95% confidence interval of the mean outcome measures were calculated for each treatment group. * The mean level chosen for all statistical tests was p = 0.05	* There was statistically significant difference with respect to histopathologic outcomes between the three treatment groups. *The thermal damage zone of the excised specimens created by the CO2 laser was significantly less pronounced than with the diode laser.
Suter V G A, Altermatt H J, Bornstein M M., *2017*	A randomized controlled clinical and histopathological trial comparing excisional biopsies of oral fibrous hyperplasias using CO_2 and Er:YAG laser.	Lasers Med Sci (2017) 32:573-581	CO_2 laser Diode laser	*IN VIVO* Excisional biopsies in oral hyperplasias *<u>CO_2 laser</u> Group 1 λ = 10.6 µm With an air-cooled Freq: 140 Hz Pulse duration: 400µs Pulse energy: 33 mJ Power: 4.62W Spot size: 0.2mm	To analyse clinical and histopathological particularities for excisional biopsies of the mucosa performed with CO_2 and Er:YAG laser in vivo. 1^o to evaluate the thermal damage zone on the excised specimens. 2^o The time of surgery, intraoperative	**Population:** * patients * only hyperplasias * length and/or height: 5mm and not exceeding 20 mm. **Specimen Preparation:** - Fixed in a 4% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 5µm; - Stained with H.E	- Software package R 3.2.2 (Viena, Austria). Fisher's exact test (for dichotomous categorical variables) - Wilcoxon-Mann-Whitney test (for continuous variables) - Wilcoxon's test (for paired groups of a continuous variable).	*Er: YAG – the lower thermal effect, tissue integrity is better preserved. *Smaller damage zone in the histopathological evaluation. *Important disadvantage: frequent bleeding and the need to apply further hemostatic methods.

				Non – contact (1-2mm distance to the mucosa) <u>* Er:YAG laser</u> Group 2 λ = 2940 nm with air-water cooling Freq: 35 Hz Pulse duration: 297 µs Pulse energy: 200 Mj Power: 7W Fiber: 400 µm. Without contact.	blleding the need for additional eletrocoagulation or sutures.	**Evaluation:** - Microscopic: No Citation **How?** - were measured in micrometres. **Where was the measurement taken?** - Measurements were done on a representative section on both lateral edge soft he fibrous hyperplasia. Zone adjacent to the epithelium of each specimen – the maximal and the minimal thermal damage.	- Significance level $\alpha = 0.05$.	

Vescovi P, Corcione L, Meleti M, *et al., 2010*	Nd:YAG laser versus traditional scalpel. A preliminary histological analysis of specimens from the human oral mucosa.	Lasers Med Sci (2010) 25:685-691.	Nd:YAG laser Scalpel	*IN VIVO* Excisional biopsies in oral lesions *Nd:YAG pulse width of 100 µs Group 1 - output power: 3.5 W; frequency: 60 Hz; fiber diameter: 320 µm (power density 488,281 W/cm2) Group 2 - output power: 5W; *frequency: 30 Hz; fiber diameter 320 µm (power density 300,000 W/cm2) Group 3 – Bard-Parker scalpel blade n. 15c.	To establish if the thermal changes induced by the Nd:YAG laser may affect the histopathological diagnosis and the evaluation of there section margins. To compare the histological features of oral benign fibro-epithelial lesions excised through Nd: YAG laser and traditional scalpel.	**Population**: *26 patients (9 men and 17 women; mean age 53 years). *26 surgical samples of benign oral lesions **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 5µm; - Stained with H.E **Evaluation:** - Microscopic 40x and 100x **How?** - The epithelial changes in proximity of the resection margin. - Connective tissue modifications (carbonization, desiccation (collagen denaturation and tissue hyalinization), presence or absence of vascular modifications and incision morphology. **Where was the measurement taken?** The edge of the margin to a depth of 1,000 µm.	Non- parametric Tests: - Chi-square test - Fisher's exact test. P< 0.05 = significant P< 0.001 = very significant P< 0.0001 = extremely significant.	*Epithelial and stromal changes were significantly more frequent in specimens with a mean size less than 7 mm (p<0.0001). *Nd:YAG laser induced serious thermal effects in small specimens (mean size less than 7 mm) *The quality of incision was better and the width of overall tissue injuries was less in the specimens obtained with higher frequency and lower power (group 1: Nd:YAG laser at 3.5 W and 60 Hz).

Wilder-Smith P, Dang J, Kurosaki T., *1997*	Investigating the range of surgical effects on soft tissue produced by a Carbon Dioxide Laser	JADA, Vol 128, May 1997.	CO_2 laser	*EX VIVO* Incisions in pig mandibles *$\underline{CO_2}$: λ= 9.3 µm Spot Size: 250 µm Duration of irradiation for each incision – 4 s Parameters: 1. CW: long pulse (1 – 1200 ms) Peak power - low and approximated average powers. 2. Superpulse mode: shorter pulses = 300 µs, Peak power – 20 W 3. Opti Pulse mode: 300 p, peak powers (60 to 100 W during any one pulse, low average powers (0.72 to 1.2 W)	To determine the range of clinical incision effects achieved in soft tissue using a wide range of laser modes and parameters at 9.3 gm. Laser effects on underlying and adjacent tissues at various soft-tissue thicknesses were also documented.	**Population:** *24 fresh mandibles * six standardized incisions * 3 centimeters in length were made in the oral * 3 incisions per parameter - parallel to the border of the mandible * 5 mm below the gingival margin **Specimen Preparation:** - Fixed in a 10% buffered formalin solution; - Embedded in paraffin block; - Sections of thickness 6 µm; - Stained with Serius Red. **Evaluation:** - Microscopic 100x **How?** Collateral tissue effects were measured at the bottom of the crater to simplify interpretation of the damage zones **Where was the measurement taken?** Measurements were performed centrally within the dot.	General linear models procedures were performed	*Incisional and collateral effects in soft tissue of CO2 laser irradiation at 9.3 gm can vary extensively *Higher average powers = increasing depths of incision *Incision width and collateral damage = results of complex interactions between the different laser parameter variables *Wide range of clinical effects can be achieved consistently and predictably in soft tissue, depending on the parameter configuration selected.

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