Journal section: Clinical Dentistry
Publication Types: Research
doi:10.4317/medoral.15.e912

Histologic evaluation of thermal damage produced on soft tissues by CO_2, Er,Cr:YSGG and diode lasers

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Received: 10/10/2009
Accepted: 14/04/2010

Cercadillo-Ibarguren I, España-Tost A, Arnabat-Domínguez J, Valmaseda-Castellón E, Berini-Aytés L, Gay-Escoda C. 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.
http://www.medicinaoral.com/medoralfree01/v15i6/medoralv15i6p912.pdf

Article Number: 16959 http://www.medicinaoral.com/
© Medicina Oral S. L. C.I.F. B 96689336 - pISSN 1698-4447 - eISSN: 1698-6946
eMail: medicina@medicinaoral.com
Indexed in:
-SCI EXPANDED
-JOURNAL CITATION REPORTS
-Index Medicus / MEDLINE / PubMed
-EMBASE, Excerpta Medica
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Abstract

Objective: The aim of this in vitro experimental study was to perform histological evaluation of the thermal effect produced on soft tissue irradiated with CO_2, Er,Cr:YSGG or diode lasers. Study design: Porcine oral mucosa samples were irradiated with Er,Cr:YSGG laser at 1 W with and without water / air spray, at 2 W with and without water / air spray, and at 4 W with water / air spray, with CO_2 laser at 1 W, 2 W, 10 W, 20 W continuous mode and 20 W pulsed mode and diode laser at 2W, 5W, and 10W pulsed mode. The thermal effect was evaluated measuring the width of damaged tissue adjacent to the incision, stained positively for hyalinized tissue with Hematoxylin-Eosin and Masson Trichrome stains. Besides, histological changes in the irradiated tissue were described using subjective grading scales. Results: The evaluated lasers developed a wide range of thermal damage with significant differences between groups. The samples with lowest thermal effect were those irradiated with Er,Cr:YSGG laser using water / air spray, followed by CO_2 and diode lasers. Conclusions: Emission parameters of each laser system may influence the thermal damage inflicted on the soft tissue, however, the wave length of each laser determines the absorption rate characteristics of every tissue and the thermal effect.

Key words: *CO_2 laser, Er,Cr:YSGG laser, diode laser, thermal effect.*

Introduction

Since the first laser device was designed in 1960, the ruby laser, many different applications of this light have been developed among medical specialties such as ophthalmology, dermatology and general surgery. At the beginning, laser use in dentistry was focused mainly on hard dental tissues, however, soft tissue surgery has been the most extended laser application throughout the past 30 years (1,2). Currently, more than ten different laser devices are available for dental application. They can be classified depending on the wavelength, the active medium, the power level or based on the biological effects that they produce.

The main difference between the scalpel and certain lasers, is the haemostatic capability of the lasers. Depending on the wavelength of the laser used, energy absorption in the irradiated tissue will be produced in a bigger or smaller tissue volume, resulting in higher or lower grade of vaporized tissue and a tissue damage area. The clinical experience gathered during the past decades discloses a number of advantages of using laser versus scalpel during soft tissue surgery, including high degree of decontamination of the surgical area, a minimum postoperative bleeding and a significant decrease of pain and inflammatory postoperative scores (3,4).

High power level lasers most frequently used in health sciences are infrared ones, which effect is based on the heat release, that is, they have an important photothermal effect. The conversion of energy into heat may produce tissue alteration, and if it has to be examined afterwards through light microscope, artifacts can appear disturbing or making difficult the histopathologic interpretation. There are several factors related to tissue that determine the damage, such as optical characteristics of the tissue, color and consistency, and those related to the laser system, wavelength and emission parameters like power, emission mode (continuous mode, pulsed mode), and power density (4,5).

The Er,Cr:YSGG laser was developed about fifteen years ago with the aim of being used in all the tissues that compose the oral cavity, oral mucosa, gingival tissue, bone, enamel, dentin and cement (6). Similar wave length laser such as Er:YAG has been described as a bactericidal effect tool as well as having a rapid wound repair on the irradiated tissue (7). The diode laser has been used in endodontics, teeth whitening and soft tissue surgery. Due to its wavelength, this light energy is not well absorbed by soft tissues, thereby producing a potential risk of deep thermal damage (3).

It is obvious that the use of a laser system that can potentially alter the results of the histopathological evaluation, cannot be considered as a reliable tool for biopsy procedures. Facing this situation, we believe it is necessary to assess which laser systems are able to make an incision in the soft tissue without the associated negative effects in the surrounding areas that could alter the histopathological examination.

The aim of this in vitro study was to evaluate the thermal damage produced by CO_2, Er,Cr:YSGG and diode lasers on soft tissue edges, examining with light microscope the extension of hyalinized or coagulated tissue adjacent to the incision.

Materials and Methods

- Sample preparation

Porcine oral mucosa samples were used to carry out this study. Specimens were irradiated within 6 hours of the animal sacrifice and stored during transit at 2-4ºC and 100 % of humidity to prevent tissue degradation. Porcine oral mucosa was selected as an experimental model due to its similarities to that to the human being. The stratified squamous epithelium of the mucosa, the lamina propia, the submucosa and the lip musculature, are proportional to those observed in the human being. Samples were obtained from the lower lip and irradiated at room temperature. Length and depth of the incision were standardized with a prefabricated template made of acrylic material, free of metal to avoid possible beam diffraction, and with a square rule shape where vertical and horizontal axis were metrically marked. A total of 13 incisions were made, 10 mm long and 5 mm depth, perpendicular to the tissue surface, all of them performed by the same experienced clinician. The emission parameters used for each laser were the recommended by the manufacturer for soft tissue surgery and some other variants selected by the investigators for the purpose of this study.

- Laser devices and parameters

Er,Cr:YSGG laser, Waterlase, (Biolase Technology, Inc., San Clemente, CA, USA) with a 2780 nm wavelength was used. The laser output power ranges from 0 to 6 W, with a non variable frequency of 20 Hz and a pulsed mode. An adjustable water-air cooling spray is provided in this device, measurable in percentage. The irradiations with this laser were performed using a handpiece with a sapphire tip and an optic fiber of 600 µm diameter at about 2 mm distance between the tip and the target tissue. Five different emission parameters were used: 1 W with 7 % of water and 11 % of air, 1 W without cooling spray, 2 W with 7 % of water and 11 % of air, 2 W without cooling spray and 4 W with 7 % of water and 11 % of air. The other laser used was CO_2 laser Sharplan 1020 (Sharplan Laser Industries, Ltd., Tel-Aviv, Israel) with 10600 nm wavelength. This laser can operate in either continuous or pulsed mode, with a maximum output power of 20 W. A straight articulated handpiece was used at a focalized distance of 120 mm from the tissue surface. The power settings used were 1W, 2 W, 10 W and 20 W in continuous mode and 20 W in pulsed mode, with 100 msec pulse width and 200 msec interval

between them. No cooling spray was available in this device. The diode laser used was the Opus 10 (Sharplan Laser Industries, Ltd., Tel-Aviv, Israel), which is a As-Ga-Al semiconductor diode laser with a 830 nm wavelength. The laser can operate both in continuous and pulsed mode, with an output power ranging from 1 to 10 W. The 300 µm optic fiber with the corresponding handpiece was used, at a pulsed mode with a 50 msec pulse width and 100 msec interval between them. The real output powers of this laser system corresponds to one third of the power reflected in the screen of the laser device due to the fact that emission time is one third of the total application time, that is, at 10 W power the real power output is 3,33 W. It was then used in a pulsed mode with 2 W, 5 W and 10 W power.

- *Histologic evaluation*

Immediately after the irradiation, the samples were sliced with at least 10 mm margin and stored in 10 % formalin solution. Samples were embedded in parafin blocks and at least 3 histologic slices of 5µm thick were sectioned from the initial, middle and final third of the block. They were conventionally stained with hematoxilin-eosin, and besides Masson thrichromic stain was used in this study to control for false positives. Overall, 78 histological preparations were obtained and evaluated for the thermal effect by measuring the extension of the hialinized or coagulated tissue adjacent to the irradiation edge. The measurements were performed using a light microscope under 40X magnification through a calibrated lenses, obtaining numeric values which represented tissue damage per metric unit and were subsequently converted into microns. All measurements were carried out by one blinded evaluator (Fig. 1).

At the same time, a photographic file was prepared, both with macroscopic and microscopic figures, in order to describe the macroscopic and microscopic morphology of the irradiated edges and evaluate them on a subjective calibrated scale.

- *Statistical analysis*

The results were analyzed by calculating the mean value and the standard deviation (SD) of the thermal effect extension for the different lasers and parameters. Differences were compared through a range variance analysis using the Kruskal-Wallis test for independent samples. The significance level for p was established at 0,05.

Results

Totally 117 measurements were performed, 9 per each laser setup studied, and their mean thermal effect and standard deviation were calculated (Table 1). Statistical-

Table 1. Thermal effect case resume, mean value and standard deviation (SD).

LASER	THERMAL EFFECT Mean (µm) ± SD
Er,Cr:YSGG	
1W water / air	9,26 ± 2,05
1W	23,38 ± 5,31
2W water / air	14,88 ± 4,09
2W	55,67 ± 17,65
4W water / air	13,42 ± 5,61
CO_2	
1W continuous	21,55 ± 8,24
2W continuous	35,16 ± 15,93
10W continuous	20,44 ± 5,43
20W continuous	29,02 ± 13,56
20W pulsed mode	20,30 ± 6,73
Diode	
2W pulsed	38,92 ± 19,92
5W pulsed	80,23 ± 3,53
10W pulsed	83,46 ± 8,38

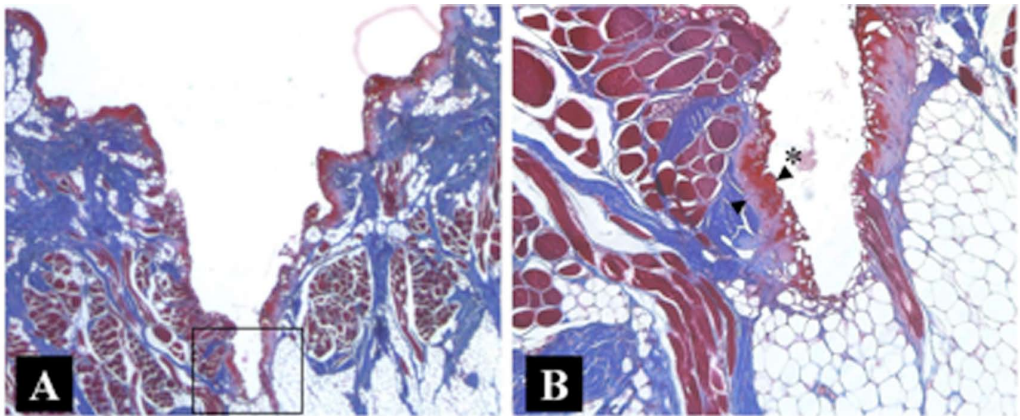

Fig. 1. Evaluated measures in the histologic preparations. A: 1,6 X General view of the incision B: 10 X, measurement of the thermal effect extension next to the incision (*).

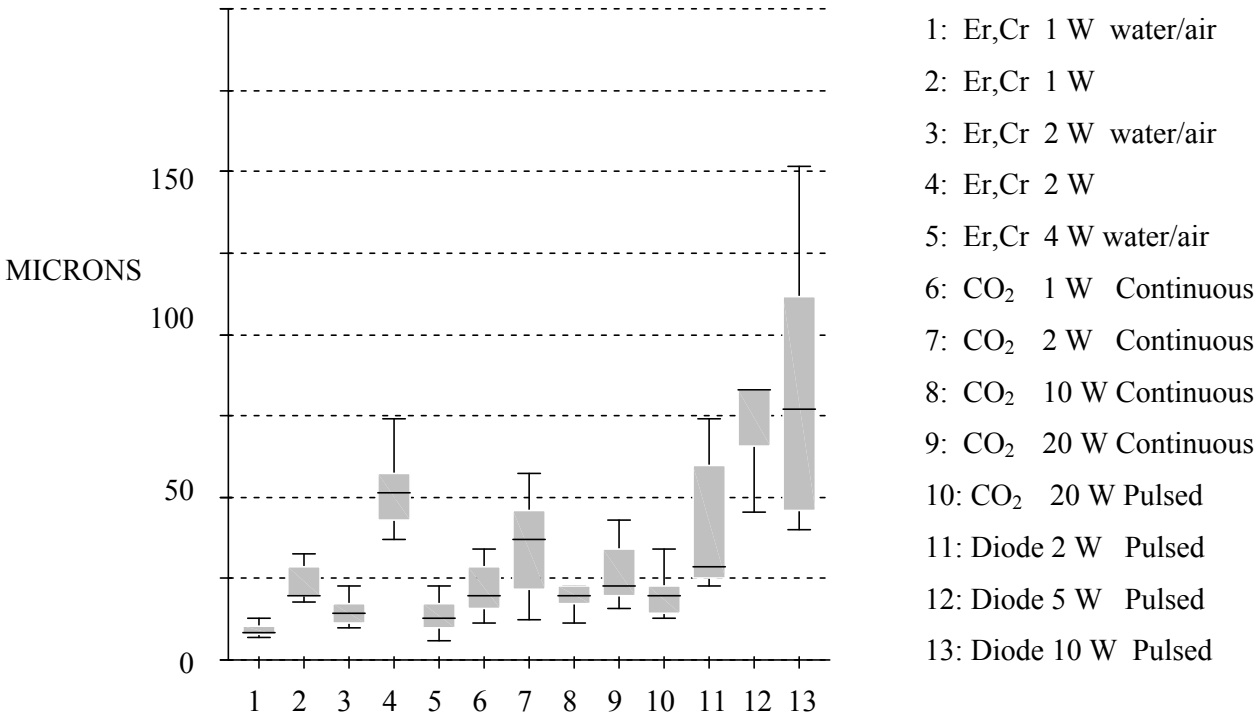

Fig. 2. Box plot showing thermal effect of the studied lasers. The measures are displayed on microns.

ly significant differences were found between the studied laser groups, with the diode laser group showing the highest scores in thermal damage extent parameter, followed by the CO_2 group and the Er,Cr:YSGG laser group. The scores obtained in the Er,Cr:YSGG group irradiated without water/air spray were significantly higher when compared to those obtained when working with the spray; thermal effect extension increased proportionally with the power in this group. The values in the CO_2 laser group, remained in the same thermal effect range for all the tested power settings (Fig. 2).

- *Macroscopic carbonization*

Macroscopically carbonization area was appreciable in all the samples irradiated both with diode and CO_2 lasers. Samples irradiated with Er,Cr:YSGG laser using water/air spray exhibited insignificant carbonization area in the soft tissue, while those subjected to irradiation of 2 W without water/air spray demonstrated a very similar morphology to samples irradiated with CO_2 laser at 2 W power in a continuous mode. At 4 W power, a negligible carbonization area of a brown-grey color was observed on the tissue surface, whereas at lower power settings non of the Er,Cr:YSGG water/air spray samples displayed evidence of macroscopic carbonization (Fig. 3) (Table 2).

- *Microscopic carbonization*

Microscopic evaluation demonstrated a varying histological anatomy of the samples, depending on the amount of connective tissue, muscular tissue or adipose tissue composing them. The adipose tissue was found to be an unreliable thermal effect indicator, since no proper and adequate staining was detected in that tissue type. Samples irradiated with CO_2 laser showed wide areas of severe pigmentation associated with a moderate carbonization area next to the incision edge, which increased proportionally with the power setting. Among Er,Cr:YSGG samples, those irradiated with water/air spray showed a small carbonization area in less than 25 % of the irradiation margin perimeter. When compared to those irradiated without the water/air spray, differences were significant, showing a moderate carbonization area. All samples irradiated with diode laser presented a wide carbonization area observable almost throughout the irradiation perimeter.

- *Artifact presence*

In the samples irradiated with diode laser, we found higher amount of cellular artifacts such as cellular hiperchromatism, intracellular vacuolization and cell structure alteration, compared to those of the Er,Cr:YSGG group. Occasionally, the artifacts in the diode groupe present in more than the 75 % of the irradiation perimeter. Samples of the Er,Cr:YSGG group showed a slight amount of artifacts surrounding the irradiation perimeter. The CO_2 group samples presented a varying amount of artifacts, which did not extent in more than 75 % of the irradiation perimeter in any case.

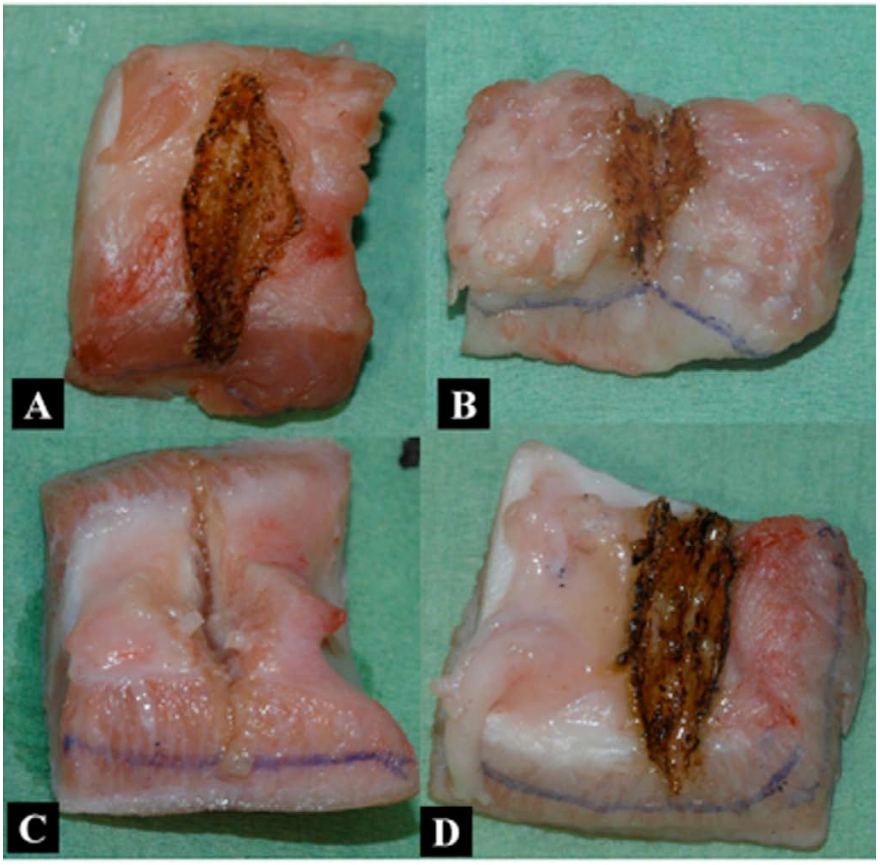

Fig. 3. Macroscopic shape of the carbonization. A: CO2 at 2 W continuous mode. B: Er,Cr:YSGG at 2 W without water / air spray. C: Er,Cr:YSGG at 4 W with water / air spray. D: Diode laser at 5 W pulsed mode.

Table 2. Tissue carbonization: macroscopic shape of the incision.

LASER	*1*	*2*	*3*	*4*	*5*
Er,Cr:YSGG					
1W water / air	X				
2W water / air	X				
4W water / air		X			
1W			X		
2W				X	
CO_2					
1W continuous			X		
2W continuous				X	
10W continuous					X
20W continuous					X
20W pulsed mode					X
Diode					
2W continuous					X
5W continuous					X
10W continuous					X

1: None
2: Brown colour on surface
3: Brown colour deeply
4: Black colour on surface
5: Black colour deeply

Discussion

The heat generated by a laser light on the tissues is difficult to assess. The literature describes different methodologies to measure it, such as measurement of temperature increase on the tissue (5,8), energy absorption by the target tissue (9,10) and the most common, the histological evaluation of the tissue next to the irradiated area (4,11,12). Most reports point out that the result of this temperature increase can be inferred from denaturalization and hyalinization of the tissue next to the irradiated area, and it can be assessed using conventional techniques of microscopy and standard staining of hematoxillin-eosin, which show an area of eosinophilic coagulation (1,12,13). In this study we used Masson trichromic staining because this technique differentiates without ambiguity thermally damaged collagen of unaffected tissue, as reported by several papers (14-16). Not only the extension of the thermal effect was objectively measured in microns, but also the degree of tissue destruction was subjectively assessed employing the description of histological artifacts and charred tissue debris in the margins neighboring the incision.

The measurements in the Er,Cr:YSGG group were lower than those reported by Rizoiu et al.(1), who found a lat-

eral hyalinized area next to the incision with a thickness between 20 to 40 µm, when using this laser in vivo with 2 W power and water/air spray in the oral mucosa of a rabbit, versus 14,88 µm in our study. These differences could be attributed to the thermal characteristics of the in vivo tissue, which can slightly modify the thermal effect, or the water content of the sample, fluctuating in different species not in terms of the overall hydration of the animal but in the difference between cellular layers. Moreover, the epithelial thickness of the pig mucosa is higher than in rabbit mucosa, attributing to a smaller size of hyalized area found in pig sample. To reproduce the body temperature, the specimen can be previously stored in an aqueous fluid at body temperature (16), but one of the most used study models, which was employed in our investigation, does not consider this variable (11).

In the present investigation, the results of the diode laser group were similar to those reported by Goharkhay et al.(4) in their in vitro study on soft tissues of a pig jaw. At 2 W power, our study showed a thermal effect of 38,9 µm versus the 40 µm of the mentioned investigation. At 5 W power, the results are practically identical, with measurements of 80,2 µm and 80 µm, respectively. Conversely, considering the results of our study, we disagree that diode laser has a tolerable thermal effect as stated Goharkhay et al. (4), since this group not only had the highest extension of the thermal effect, but also the histological descriptions disclosed a clear predisposition of the preparations to have extensive areas of charring and unidentifiable artifacts.

Wilder-Smith et al. (5) in 1995 studied the influence of the wavelength of the CO_2 laser on the ability to do incision in soft tissues and its lateral thermal effect. One of the investigated devices, with a wavelength of 10600 nm, was the same that we used in the present study. Although the power settings were different between studies, 4 W versus 2 W power in our study (both in pulsed mode), the thermal effects were comparable and measured 54,6 µm and 35,1 µm respectively.

All studied lasers, except for the CO_2 group, had an increase of thermal effect, which was directly proportional to the power. In the CO_2 group, however, the depth of the thermal effect was maintained from 2 W up to 20 W power, which could explained by the fact that the necessary working time with low power to make a standardized incision is higher than with more power, causing a higher concentration of heat and thermal effect on the tissues, if the time of thermal relaxation is not respected.

The subjective scales were of great value to show that, despite having similar measurements of the extension of the collateral thermal effect in different groups, the histological images were clearly different. Macroscopically, all samples had some degree of charring, with the exception of the Er,Cr:YSGG samples with water/air spray at 1 and 2 W. Microscopically, the presence of debris of charred tissue was clearly visible in all samples but remained minimal in the Er,Cr:YSGG group with water/air spray. From lower to higher degree, the order was: Er,Cr:YSGG with water/air spray, Er,Cr:YSGG without water/air spray, CO_2 and, finally, the diode laser group, where charring was present in most of the perimeter of irradiation. There was also a directly proportional relationship of the lateral artifacts on the irradiation margins (defined as histological changes such as intracellular vacuolization, cellular hyperchromatism and loss of intracellular structure) with the degree of charring of the tissues, pinpointing the diode group as the one causing the highest number of cytological artifacts.

Classical surgical procedures using laser include not only those that require tissue ablation with charring and vaporization, such as frenectomies and gingivectomies, but also those that require the total removal of the lesion, such as the excision of exofitic lesions or flat lesions of limited size. In this cases, it is strictly compulsory to do a histological examination to diagnose benignity. Moreover, the removal-biopsy with laser must allow, in case of a diagnosis of malignity, to define with clarity whether the margins of the histological specimen are disease-free or not. The pathologist will search the margin to disclose mitosis, hyperchromatism and other characteristics of cellular dysplasia, and the mentioned artifacts can easily confuse or alter the evaluation of the margins of the removed tissue. It continues to be one of the most imperative concerns when using lasers to perform biopsies.

Although a considerable body of literature describes the distortion caused by a laser in the examination of histological samples, few references describe the types of lasers that are adequate to perform biopsies, without rendering difficult the diagnosis of the pathologist. Moreover, it is challenging to compare across studies because emission parameters are usually different. Eversole (17) reported that after 6-years experience in soft tissue surgery with laser, the Er,Cr:YSGG system is comparable to conventional scalpel when it comes to histological evaluation, whereas CO_2 and Nd:YAG lasers create cytological artifacts, such as hyperchromatism, pleomorphism and nuclear elongation, that can cause diagnostic confusion. Conversely, other authors (18-20) stated that when employing the CO_2 laser it is hardly possible to make enough artifacts to invalidate the histological examination, if the laser is not deliberately misused. Both authors agree on the importance of the emission parameters to minimize thermal damage and occurrence of artifacts. Based on the results of this study and our experience, we feel that, generally, the thermal effect of the laser does not undermine the histological diagnosis of a lesion, since the remaining tissue situated outside of the irradiation margin is not damaged and can be properly diagnosed. However, if

disease-free margins are needed, the pathologist could encounter serious difficulties to evaluate them due to the presence of charred tissue, artifacts and denaturalized, coagulated and disorganized tissue of variable extension around the margins.

Differences in the thermal damage next to the incision have been detected among the evaluated lasers in this study. A great range of photothermal effects between diverse combinations of laser parameters have been previously reported by several authors (5,17). Rizoiu et al. (1) studied the effect of Er,Cr:YSGG laser in an animal model, New Zealand white rabbit, and demonstrated that the laser-operated group had hyalinization and coagulation at the incision margins. Likewise, no morphological changes were observed in the adjacent cells of the Er,Cr:YSGG group in comparison with the scalpel-operated group. These authors recommend the use of laser for tissue removal when a biopsy is needed, based on its low thermal effect, the minimal bleeding and the absence of artifacts. Our results in the Er,Cr:YSGG group with water/air spray coincide with those described by Rizoiu et al. (1) Histological preparations disclose an apparently healthy tissue with limited areas of denaturalized tissue that were 10-fold smaller than in the diode laser group. This is probably due to the refrigeration capacity of the water/air spray, because the samples of the Er,Cr:YSGG without water/air spray had an extension of the thermal effect similar to the CO_2 group.

Although the CO_2 laser produced a considerable thermal effect, it is necessary to take into account that, from the physical point of view, the thermal effect is due to an excess of accumulation of heat in relation with the time of action of the energy. Soft tissues transmit heat badly because they had a high time of thermal relaxation. The CO_2 laser, however, compared to the diode laser, has a high coefficient of absorption by water, which fosters surface absorption in soft tissues and a comparatively reduced thermal effect on adjacent tissues.

To minimize the thermal effect on tissues and in order to obtain the therapeutic effect with the lowest irradiation time, the power density, the continuous or pulsed mode, the duration of pulse and, pause interval must be set at adequate values.

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