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Evaluation the Effects of CO_2 Laser on Soft and Hard Tissues (in vitro study)

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Abstract: CO_2 laser (10.6 µm) is the most often used laser in the oral surgery due to its high absorption by water of the oral tissues. Several benefits of the use of CO_2 laser have been reported for oral surgical procedures. This study aims to evaluate the effect of CO_2 laser on soft and hard oral tissues (in vitro study). This study was done on fresh tissues from sheep's head. CO_2Surgical Laser with different operation modes was used; 0.2 mm spot size using different laser parameters on the tongue, and bone making holes, incisions and cutting. The depths and widths of holes and incisions were measured using endodontic file under magnification. The speed of incisions was calculated and the required time for cutting was measured using sport clock. The depths of holes and incisions were increasing with increasing the power density and pulse duration from 90 µs to 1.7 ms in 100 µs increments and so in CW mode (for holes 2.5 mm at 15W, for incisions 6mm at 20W). Also the diameter of holes was increasing with power (1.2 mm at 15W) .The required time for cutting was decreasing as the power increased (14.14 sec. at 25W). The use of CO_2 laser can be considered practical, effective and easy to carry out holes، incisions, and cuttings.

Introduction

Since the development of the first laser in the 1960's, dental researchers have investigated the effects of laser radiation on teeth, bone, pulp, and oral mucosal tissues.CO_2 laser 10.6 µm is absorbed strongly in water in oral tissues. Excellent incisional and ablational effects can be achieved. The Food and Drug Administration (FDA) granted clearance for marketing CO_2 lasers for soft tissue procedures such as frenectomy, gingivectomy, biopsies, and removal of benign and malignant lesions. Specific indications for use in dentistry include apthous ulcer treatment, coagulation of extraction sites, sulcular debridement, and intraoral soft tissue surgeries such as ablating, incising, and excising. The benefits of using laser versus scalpel during soft tissue surgery, include relatively bloodless surgery with little if any bleeding post-surgery; site-specific wound sterilization; minimal swelling and scarring; reduced necessity for suturing; decreased incidence of mechanical trauma; shorter operative time; favorable patient acceptance; decreased use of local anesthesia; and little or no post-operative pain (Vaderhobli, et al 2010).

The clinician should be able to predict and achieve specific incisional or ablational effects. The required range of incisional effects in soft tissue is extensive. Wide or narrow, deep or shallow incisions may be indicated; alternately, fast large-surface ablation or microsurgical finesse may be desirable. Laser effects on adjacent and underlying tissues such as bone or tooth must be considered, as there may be specific limits regarding collateral or thermal effects. Finally, there should be easy access with the laser device to any part of the mouth (Wilder-Smith and Dang, 1997).

Aim of Study was to evaluate the effect of CO_2 laser on soft and hard oral tissues using different laser modes and parameters at 10.6 µm in vitro model.

Materials and Methods

Sample Preparation: This study was done on tongue, and bone from a fresh sheep's head (within 24 hours of animal sacrification from a local slaughter).The tissue specimens were stored in a refrigerator. Specimens were allowed to return to room temperature before experiments were conducted.

Laser Device and Parameters: We used a CO_2 laser with 10.6 µm in different modes of applications (ultra dream pulse surgical CO_2 laser system, DS-40U, DAESHIN ENTERPRISE Co., Ltd., Korea); the laser was delivered via an articulating arm, the beam was focused by hand-piece with a focal length of 50 mm, and a wooden tongue depressor was used to measure spot size (0.2mm).

In this study, the used parameters were belonged to the following modes:

1. The Continuous Wave Mode: A continuous laser beam is radiated within the output range of 1W-40W in 1 W steps.
2. The Super Dream Pulse Mode: This mode can adjust pulse duration (1ms-1.7ms) in 0.1ms steps and the peak power during each pulse is changing accordingly (177W-146W), in single or repeated pulse with a repeat time (2ms-500ms).
3. The Ultra Dream Pulse Mode: This mode can adjust pulse duration (90µs-900µs) in 100µs steps and the peak power during each pulse is changing accordingly (315W-188W), in single or repeated pulse with a repeat time (2ms-500ms).

Procedures: Irradiation was done perpendicular in contact with the tissue specimens at focusing. Different laser parameters were used in each irradiation episode. The time was measured using sport clock (sportline, model 410, China).

Soft Tissue Holes: Tongue was divided into right and left halves and marked with (54) points. 54 holes (24 holes in Super Dream Pulse Mode and 30 holes in Ultra Dream Pulse Mode), were performed, 3 holes per each pulse duration at pulse intervals 50ms, 70ms, and 100ms. The tip of the hand-piece was held stationary and adjacent to the marked points for 5.338 seconds average operating time. The depths of the holes were measured using endodontic file size 55.

Hard Tissue Holes: 10 holes (using CW mode only) were performed in bones. The tip of the hand-piece was held stationary for average operating time 5.54 seconds. The depth of the holes was measured using endodontic file size 60, and diameter of holes, carbonization, and whitish halos were measured under light disecting microscope (Bausch and lomb, Germany) 14x magnification power.

Soft Tissue Incisions: Sheep's tongue was marked with 10 lines of 1cm in length. CW irradiation was done adjacent to the marked lines in one movement along the line to incise it.

Nine lines of 1 cm in length were irradiated by 10 repeated passes along the line (Super Dream Pulse Mode), 3 incisions for each fluence were performed at pulse intervals 30ms, 50ms, and 100ms.

Depths, width of the incisions (using endodontic file size 80) were measured. The tissue carbonization degree was noticed. The time required was measured.

Soft Tissue Cutting: A piece of tongue (2.5cm length, 2cm width) was cut using 15W and 25W in CW mode. The technique was done by moving the hand-piece continuously. The required time for cutting was measured using sport clock.

Hard Tissue Cutting: Different laser powers (CW mode) were used in each irradiation episode to cut the bone (12.5mm length) completely with repeated passes. Time required for cutting was measured, and the speed was calculated.

Results

The morphological changes & measurements were studied with gross observation, and light microscope.

Soft Tissue Holes: 54 holes on tongue, 3 holes per each pulse duration were performed at 50ms,70ms,100ms pulse intervals .24 holes in Super Dream Pulse Mode and 30 holes in Ultra

Dream Pulse Mode. The holes were circular in shape. The depth of holes was increasing with increasing the pulse duration from 90 µs to 1.7ms in 100 µs steps. In The Super Dream Pulse Mode: The depth of the holes was measured (table 1). At pulse interval 50ms, Brown walls can be easily noticed, which were more than of holes at pulse intervals 70ms, and 100ms. The diameter of holes at fluences 140.923 J/cm^2 and 162.738 J/cm^2 was 1mm while diameter of the other fluences was 0.5mm.

Table (1) : Super Dream Pulse Mode, the tongue holes depth at different fluences and pulse intervals, for 5.383 sec. average operating time

Fluence (J/cm^2)	Pulse width (ms)	Depth (mm) At Pulse Interval		
		50 ms (average operating time (5.40 sec.))	70 ms (average operating time (5.42sec.))	100ms (average operating time (5.33 sec.))
140.923	1	2	2.75	2.75
148.885	1.1	2.5	2	4
156.289	1.3	2.5	3.5	4.25
158.598	1.2	3	2.75	3
162.738	1.4	3.25	4	4.5
174.363	1.5	2.75	3.5	3.5
185.987	1.6	2.5	3	3.75
197.611	1.7	2.25	3.25	3

In the Ultra Dream Pulse Mode, the holes were less in depth than that of the Super Dream Pulse Mode. The depths of the holes were measured Table 2.

Table (2) : Ultra Dream Pulse Mode, the tongue holes depth at different fluences and pulse intervals, for 5.293 sec. average operating time

Fluence (J/cm^2)	Pulse Width (µs)	Depth (mm) At Pulse Interval		
		50 ms (average operating time 5.26 sec.)	70 ms (average operating time 5.35 sec.)	100ms (average operating time 5.27 sec.)
22.571	90	2.875	1.75	1.5
23.168	100	2	2.5	1.75
44.745	200	1.875	2.5	2
64.490	300	2.5	2.25	2
83.121	400	1.5	2	2.875
100.318	500	2	2	2.5
118.471	600	2.75	2.25	2
134.315	700	2.5	2.5	2.625
140.127	800	2.6	2.5	2.25
134.713	900	3	2.55	2.25

Hard Tissue Holes: The focused beam produced 10 holes (1 hole per each power in a CW mode) and a cloud of smoke. The depths of the holes and the diameters of the holes, carbonization and whitish halos were measured (table 3).The depth of holes and the diameter of the thermal affected zone were increasing with increasing the power density figure(1).The holes were cone in shape with the widest area at the orifice except the holes at 11146.4 W/cm^2 and 11942.6 W/cm^2 were cylindrical in shape.

Table (3): CW Mode, depth and diameter of the bone holes, for 5.54 sec. average operating time.

Power Density (W/cm^2)	Operating Time (sec.)	Depth (mm)	Diameter (mm)		
			Hole	Hole and carbonization	Hole ,carbonization and whitish halo
2388.5	5.49	0.5	0.2	0.9	1.1
3980.8	5.46	2.5	0.6	1.2	1.5
5573.2	5.45	3	0.7	1.4	1.6
7165.6	5.68	2.25	0.9	1.5	1.9
7961.7	5.43	2.5	1	1.5	2
8757.9	5.51	2.6	1.1	1.6	2.2
9554.1	5.60	2.5	0.9	1.4	2.5
10350.3	5.43	2	0.9	1.6	2.8
11146.4	5.89	2.5	1	1.9	2.9
11942.6	5.39	2.5	1.2	2.1	3.3

Fig. (1): Hard tissue holes (CW Mode).

Soft Tissue Incision: 19 incisions (1 cm length) were performed, 10 incisions in CW Mode and 9 incisions in Super Dream Pulse Mode.

CW Mode clinically provided a rapid, clean incising effect figure (2). Depths and the widths of the incisions, and tissue carbonization degree, were measured (table 4).The depths and the widths of the incisions and the tissue carbonization degree were increasing with increasing the power density.

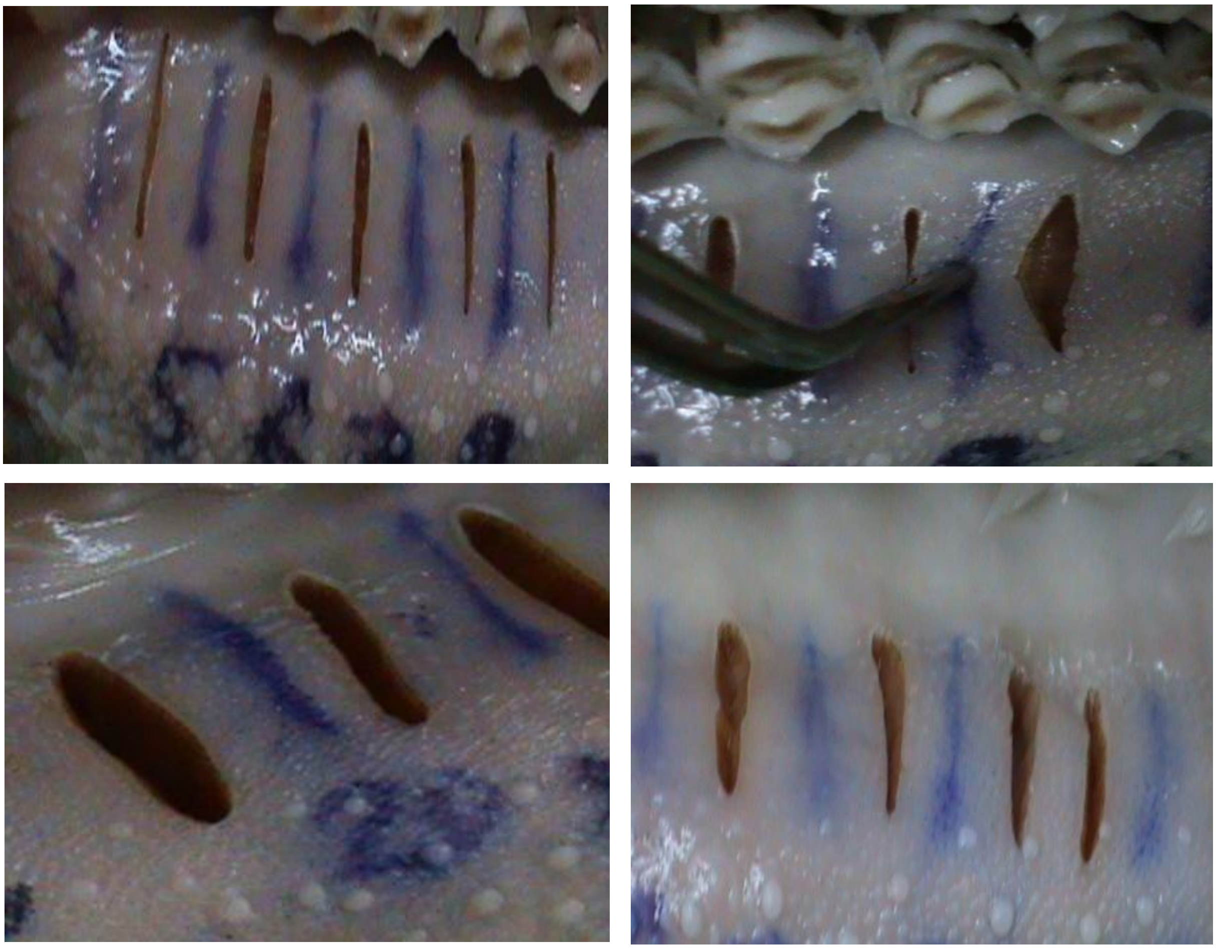

Fig. (2): Tongue incisions (CW Mode).

Table (4): CW Mode, the depth, width and tissue carbonization scale of the tongue incisions, at 2.174 mm/sec. average speed.

Power Density (W/cm^2)	Operating Time(sec.)	Depth(mm)	Width(mm)	Carbonization scale*
2388.5	6.52	0.75	0.5	0
3980.9	6.39	2	1.5	1
4777.1	4.33	2.5	1.5	2
5573.2	4.06	3	1	2
7165.6	4.20	4.5	1	2
7961.8	3.89	4	0.75	2
9554.1	3.57	4.5	3	2
11942.7	5.14	5.5	3	2
14331.2	3.37	5	2	2
15923.6	4.57	6	3.5	2

*Carbonization scale:

0 None (pink color)
1. Light Brown color
2. Brown color
3. Dark Brown color
4. Black color

Super Dream Pulse Mode produced clean, V-shaped incisions on the tongue figure (3), 3 incisions per each pulse interval at 3 different fluences, but they were less in depth and width than those of CW Mode.

Fig. (3): Tongue incisions (Super Dream Pulse Mode).

The depths and the widths of the incisions, and tissue carbonization degree were measured (table 5).The depth of the incisions was increasing with increasing the fluences, and decreasing with increasing the pulse interval, while the tissue carbonization degree was increasing with decreasing the pulse interval. At pulse interval 50ms, incisions produced no visible signs of carbonization.

Table (5): Super Dream Pulse Mode, the depth, width and tissue carbonization scale of the tongue incisions, at 5.391 mm/sec. average speed.

Fluence (J/cm^2)	**Pulse Width (ms)**	**Pulse interval (ms)**	**Operating Time(sec.)**	**Depth (mm)**	**Width (mm)**	**Carbonization scale**
140.9	1	30	17.67	1.5	1	1
140.9	1	50	17.94	2.25	1.5	0
140.9	1	100	18.19	0.25	0.5	1
162.7	1.4	30	18.21	2	1.5	2
162.7	1.4	50	16.79	1	1	0
162.7	1.4	100	17.30	0.5	0.5	1
197.6	1.7	30	19.12	3.5	1	3
197.6	1.7	50	17.75	0.875	2.25	1
197.6	1.7	100	15.45	0.5	0.5	1

Soft Tissue Cutting: Cutting of tongue muscle at 11942.67 W/cm^2 and 19904.45 W/cm^2 in CW mode resulted in brown color on cut surface and black color on incised surface. A Tongue piece of 2.5cm length, 2cm width &1.5cm thickness, was cut in 78.26 second at 19904.45 W/cm^2 , Figure(4).

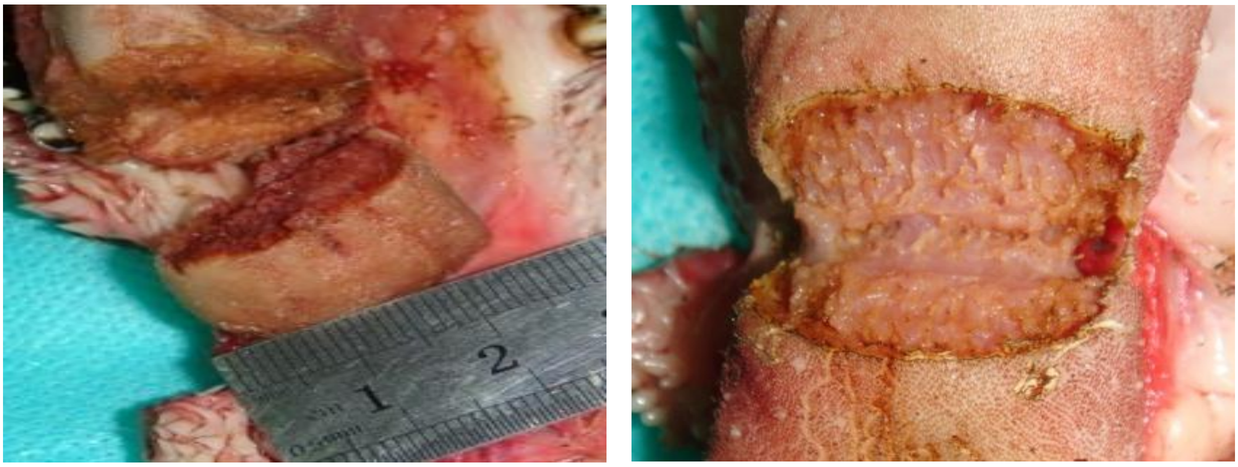

Fig. (4): Soft tissue cutting (CW Mode).

Hard Tissue Cutting: Different laser powers (only CW mode) were used to cut the bone. The required time for cutting was decreasing with increasing the power density. While the widths of cutting, were not significantly related to the used power densities (Table 6).

Table (6): CW mode, the width and the required time for bone cutting, at 5.33mm/sec. average speed.

Power Density (W/cm^2)	**Thickness(mm)**	**Length(mm)**	**Width(mm)**		**Required time (sec)**
			Anteriorly	Posteriorly	
3980.9	1.45	12	1.5	1.25	96.92
7961.8	1.6	12.5	1	0.75	43.43
11942.7	1.6	13			36.63
11942.7	1.65	12.5			42.59
11942.7	2.4	12.5	1.5	0.75	55.77
15923.6	2.45	12.5	2	0.75	33.14
15923.6	2.4	14.5	1.75	1.1	29.20
19904.5	2.45	12.5	1.5	0.75	14.14

Discussion

Medical applications of lasers have focused particular interest on the interaction of infrared laser light in biological tissue. The physical heterogeneity of biological tissue results in considerable regional variation in light absorption and scattering, two of the critical processes that determine the pattern of heating (Cummins, and Nauenberg, 1983).

This study determined the safety and effectiveness of an ultra-dream pulse surgical CO_2 laser. The study confirmed the microsecond pulse duration and small spot size of delivery system of this laser device and determined its effects in an in vitro model. The model utilized tongue and bone in vitro, are the standard for testing the cutting efficiency of dental lasers.

The aim of this study was to describe the specific effects of various powers, pulse width and pulse interval on soft and hard oral tissues, in order to apply the newer microsecond pulse CO_2 laser technology in the safest, most efficient manner.

There are several factors related to tissue such as optical characteristics of the tissue, color , consistency and thermal tissue properties (heat capacity, temperature conductivity), and those related to the laser system, wavelength and emission parameters like applied power, focal spot size, and emission mode (exposure time), that determine the effect of laser on the tissue (Cercadillo- Ibarguren, *et al.*, 2010).

Carbon dioxide laser irradiation at a 10.6 µm wavelength is almost completely absorbed by the water in the tissue, ensuring minimal thermal damage and spread, and is directly converted into heat energy (kinetic energy of water molecules).The generated heat inside the exposed tissue is a function of intensity and absorption coefficient which is strongly wavelength-dependent. This heat energy produces tissue alterations and rapid vaporization of the tissue water component only in the immediate vicinity of the laser beam, and all tissues are uniformly affected by the first 90µm.of tissue. The generated vapor carries away excess heat and helps to prevent any further increase in the temperature of adjacent tissue. This makes the CO_2 laser particularly well suited for use near critical anatomical structures (Hanby, *et al*, 2011, Markolf Neimz 2007)

The increase in temperature proceeds as laser exposure is continuing, reaching carbonization that should be avoided in medical laser applications, since tissue already becomes necrotic at lower temperatures, increasing thermal side effects on adjacent tissues and delay healing. The ablation spatial extent primarily depends on magnitude, exposure time, and placement of deposited heat inside the tissue (Markolf Neimz 2007).

The effect of the CO_2 laser on different types of tissue varies depending on differences in water content and soft tissue density, with a wider necrotic band in dense fibrous tissue and muscle and a shorter one in salivary glands and loose connective tissues (Seoane, *et al*, 2010).

Incision depth and width correlated strongly and positively with power density. This result is logical and is confirmed by the results reported from Wilder et.al study (Wilder-Smith P. and Dang, 1997).

Incision shape and width were also strongly mode-dependent. The CW Mode produced relatively deep and wide, almost straight-sided incisions. Thus, this mode can cut or ablate large amounts of tissue quickly and effectively in a controlled fashion. However, the proximity of underlying or adjacent bone or other heat-sensitive structures must be considered. Laser incisions directly over or adjacent to heat-sensitive tissues are better performed with the Super Dream Pulse Mode.

In the Super Dream Pulse Mode, very narrow, shallow incisions were achieved than those using CW mode. No carbonization was observed .These effects are attributed to the short pulses (1 ms,1.4 ms, 1.7 ms) and high peak powers (146,177 W), permitting good incisional effects while minimizing heat transport into adjacent structures.

Thus, this laser configuration is well-suited to applications requiring skilled surgical finesse, or where the properties of adjacent and underlying tissues stipulate minimal thermal disturbance.

Incisions comparable in depth to those achieved using the CW mode, were completed efficiently at lower average powers with the Super Dream Pulse Mode.

The lack of carbonization and its sequels observed in our samples at pulse duration of (1-1.7ms) is confirmed by Walsh and colleagues (Walsh, and Flotte, 1988), who reported no charring after soft-tissue incision at pulse durations less than 2 ms. Similar results have been reported by other authors (Wilder-Smith and Dang, 1997), and are attributed to the much shorter pulses.

Although the CO_2 laser produced a considerable thermal effect, the used technique was done by moving the hand-piece continuously, because it is necessary to take into account that, from the physical point of view, the thermal effect is due to an excess accumulation of heat in relation with the time of action of the energy i.e. the rate of heat transport is less than the rate of heat generation, since soft tissues transmit heat badly because they had a high thermal relaxation time. Therefore there is a dependence of temperature on repetition rate of laser pulses. A high repetition rate can evoke an additional increase in temperature resulting in more thermal damage to the adjacent tissues.

However, when the proper technique and laser dose parameters are used, no evidence of tissue carbonization is seen. It is theorized that inadvertent exposure would produce similar results clinically. This is important to avoid surface charring when performing soft tissue surgery around teeth.

As the pulse duration increases, so do the interaction time and thermal effects. Based on the depth of cutting data mentioned, clinicians are encouraged to use the minimum dose parameters to achieve the desired effect.

Even without detailed thermal model, however, the fact that we detected clinically no cracks in bone caused by thermal or mechanical stresses, along with the confinement of carbonization effects to the ablation crater alone, is very encouraging. This suggests that with proper parameter selection, CO_2 laser surgery in hard tissue can be accomplished safely while achieving the desired effects, and this is in agreement with Wong's study (Wong, Jon lee, *et al*., 1997).

Although nothing specific was done to limit or control the thermal side effects of CO_2 laser, the system had a low volume air flow at the hand-piece tip primarily to keep ablation products from entering and clogging the tip and smoke evacuator system have provided an additional air breeze that contribute as a coolant decreasing the collateral thermal damage of adjacent tissues and clearing the field of debris, and plume and this is in agreement with Hanby's study (Hanby, *et al*, 2011).

In this study, laser incisions were done to manually simulate the clinical settings. Using an in vitro model, sheep's tissues, has its own advantages in being able to evaluate many laser dose parameters including high power, which would not be utilized in vivo.

In order to measure the inflammation and healing events, an in vivo work must be utilized. It is preferable to utilize an in vitro model first to determine laser parameters and evaluate the tissue effects that are suitable for clinical applications.

If dentists are to use lasers effectively and safely, they must be able to achieve a wide range of incisional and ablational effects predictably and consistently. In the mouth, for example, surgical needs in soft tissue include large-area ablation of superficial structures such as benign, malignant or vascular lesions without adverse effects to underlying bone. Alternately, narrow, deep incisions with minimal lateral effects may be required, perhaps for periodontal surgery in close proximity to teeth. A microsurgical capability to achieve finely controlled microincisions with minimal collateral thermal effects might be appropriate. As surgeons, we are all on a perpetual search for a perfect cutting tool. The ideal instrument would accomplish the necessary functions of cutting and coagulation while minimizing collateral tissue damage as in laser-assisted frenectomy.

Conclusion

All laser parameters studied are able to reach the defined simulation objectives in reasonable amounts of time, less than a minute. The use of higher power densities correlates with increasing depths of incision and holes. The results of this study demonstrate that a wide range of clinical effects can be achieved consistently and predictably in oral surgery, depending on the selected parameter configuration. 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, pulse interval must be set at adequate values. In conclusion the use of CO_2 laser can be considered practical, effective and easy to carry out incisions, cuttings and holes.

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تقييم تأثير ليزر ثاني أوكسيد الكاربون على الانسجة الرخوة و الصلبة (دراسة خارج الجسم الحي)

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الخلاصة: ليزر ثاني أوكسيد الكاربون (10.6مايكرومتر) هوالاكثر استخداما" في جراحة الفم ،وذلك لامتصاص ماء أنسجة الفم العالي له.لاستخدام ليزر ثاني أوكسيد الكاربون فوائد عديدة قد تم اقرارها في عمليات جراحة الفم. هو تقييم تأثير ليزر ثاني أوكسيد الكاربون على انسجة الفم الرخوة والصلبة (دراسة خارج الجسم الحي). في هذه الدراسة أستخدمت أنسجة طازجة مأخوذة من رأس خروف و ليزر ثاني أوكسيد الكاربون الجراحي ذو صيغ تطبيقية مختلفة ، بنصف قطرحزمة (0.2مليمتر) بمعايير جرع ليزر مختلفة،على اللسان و العظم .لعمل ثقوب و شقوق وتقطيع. تم قياس عمق و عرض الثقوب و الشقوق باستخدام فايل حشوة الجذر(آلة قياس) تحت التكبير.وتم حساب سرعة عمل الشقوق وقياس الوقت اللازم لاكمال التقطيع بأستخدام ساعة رياضية. كانت أعماق الثقوب و الشقوق تزداد بأزدياد كثافة القدرة و عرض النبضة في الطراز النبضي (من 90مايكروثانية الى 1.7ملي ثانية بخطوات100مايكروثانية) وكذلك في طراز الموجة المستمرة (للثقوب 2.5مليمتر عند15 واط،وللشقوق 5مليمتر عند 20واط)،وكذلك قطر الثقوب كان يزداد بأزدياد كثافة القدرة (1.2مليمتر عند 15 واط)،أما الوقت اللازم لاكمال القطع كان يقل بأزدياد القدرة(14.14 ثانية عند 15 واط). أن أستخدام ليزر ثاني أوكسيد الكاربون من الممكن أعتباره عمليا" و فعالا" و سهلا" في أجراء الثقوب و الشقوق و التقطيع.