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Authors
Wilder-Smith, Petra
Dang, Jennifer
Kurosaki, Tom
et al.

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The influence of laser parameter configurations at 9.3 µm on incisional and collateral effects in soft tissue

Petra Wilder-Smith, BDS (Hons), LDS, RCS (Eng), D.M.D.,[a] Jennifer Dang, BS,[b]
Tom Kurosaki, MS,[c] and Joseph Neev, PhD,[d] Irvine, Calif.
BECKMAN LASER INSTITUTE AND MEDICAL CLINIC, UNIVERSITY OF CALIFORNIA, IRVINE

These investigations were performed to determine histologic and incisional consequences of varying pulse duration, duty cycle, and average powers during laser incision at 9.3 µm in soft tissue. In 19 fresh pigs' jaws six standardized incisions 3 cm long were made per parameter with a template and motorized jig. Laser parameters investigated were average power: 1 to 9 W, duty cycle: 10% to 80%, and pulse duration: 1 to 200 msec. The gated Cw mode was used. Incision width and depth and collateral tissue effects were assessed statistically with general linear procedures. Multiple factors were found to influence the outcome of laser irradiation. Depth of incision correlated positively with average power. Tissue damage correlated strongly and negatively with all three variables. These results demonstrate that a wide range of surgical and collateral effects can be achieved with one specific laser device depending on the parameter configuration selected. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997;84:22-7)**

In the CO_2 lasers traditionally available to clinicians, light at 10.6 µm is delivered by means of an articulated arm or a hollow waveguide and a handpiece to the surgical site. Many areas of routine CO_2 laser use for soft tissue surgery have developed over the past 30 years including orofacial surgery and periodontal applications. Clinical and laboratory investigations have consistently confirmed the advantages of this tool: precision, minimal intraoperative hemorrhage, sterilization of the surgical area, and healing with minimal scarring, postoperative pain, and swelling.[1-5]

Recently, CO_2 lasers that deliver light in the 9.3 µm region of the infrared spectrum through a coherent flexible beam delivery system at a wide range of laser parameters with potential for tailoring parameter combinations to individual clinical situations have been developed. Recent studies have demonstrated very similar incisional, thermal, and histologic effects in soft tissues of the two wavelengths.[6] In addition, a wavelength of 9.3 µm better matches the absorption characteristics of hydroxyapatite than 10.6 µm, providing the possibility for modification[7] or efficient ablation of hard dental tissues without thermal damage to adjacent and pulpal structures. Because lasers are relatively costly devices, multiple applications are desirable if this type of equipment is to become realistically useful for clinicians.

The laser energy applied to the target tissues will also affect to a varying degree adjacent or underlying tissue structures. The extent of collateral damage is related to the absorption characteristics of light in the tissues and to the laser parameters and beam configurations used. For most clinical applications the zone of thermal damage to adjacent structures should be kept to a minimum, because it may impede wound healing and graft take and reduce wound tensile strength. Furthermore laser-induced temperature increases, related, for example, to long pulse durations in some situations, can threaten the vitality of adjoining structures such as teeth, pulp, or periodontium. Conversely, very short pulse durations used to minimize collateral thermal effects may hinder hemostasis and reduce the ease of incision or ablation; attempts at compensating for reduced cutting efficiency by increasing pulse frequency can result in an exacerbation of collateral thermal effects.[8] Thus the configuration of laser parameters used in a specific clinical setting must be carefully balanced to optimize the desired effects while minimizing collateral implications.

The aim of these investigations was to determine the consequences of varying pulse duration, duty cycle ([laser on]:[laser on + laser off] ratio), and power of laser irradiation at 9.3 µm on histologic and incisional effects in intraoral soft tissues.

MATERIAL AND METHODS

In 19 fresh pigs' mandibles six standardized incisions 3 cm long were made in the oral mucosa per laser parameter combination. A total of 19 laser configurations were used (Table I). A template was positioned 3 mm below the planned incision site during the performance of each incision. The laser handpiece was attached to a motorized slide to standardize the incision and precisely control variable movements; the pigs' jaws were immobilized on a veterinary mount (Fig. 1). Three inci-

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[a]Director of Dentistry and Assistant Professor.
[b]Researcher. Principal Statistician,
[c]Epidemiology Division, Department of Medicine.
[d]Assistant Professor.

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Table I. Incision depth and width, vertical and horizontal damage measured in micrometers

Average Power (W)	*Pulse width (msec)*	*Pulse repetition per second (Hz)*	*Incision depth* μ*m ± S.D.(n)*	*Incision width* μ*m ± S.D.(n)*	*Vertical damage* μ*m ± S.D.(n)*	*Horizontal damage* μ*m ± S.D.(n)*
1	200	0.5	835.50 ± 21.70 (15)	355.00 ± 12.40 (15)	51.20 ± 7.75 (15)	62.00 ± 0.00 (15)
1	200	1.5	681.90 ± 15.50 (30)	358.10 ± 26.40 (30)	66.70 ± 14.00 (30)	102.30 ± 12.40 (30)
1	200	4	358.10 ± 57.40 (30)	348.80 ± 37.20 (30)	34.10 ± 21.70 (30)	60.50 ± 15.50 (30)
3.5	200	1.5	1037.00 ± 108.50 (30)	446.40 ± 41.90 (30)	54.30 ± 14.00 (30)	77.50 ± 7.75 (30)
3.5	200	2.5	457.30 ± 7.80 (30)	488.30 ± 23.30 (30)	45.00 ± 4.70 (30)	77.50 ± 0.00 (30)
3.5	200	4	327.10 ± 15.50 (30)	372.00 ± 21.70 (30)	46.50 ± 6.20 (30)	72.90 ± 6.20 (30)
9	200	4	1075.70 ± 20.20 (30)	313.10 ± 17.10 (30)	40.30 ± 9.30 (30)	46.50 ± 0.00 (30)
1	20	5	717.70 ± 63.60 (30)	593.70 ± 35.70 (30)	100.80 ± 18.60 (30)	153.50 ± 6.20 (30)
1	20	15	525.50 ± 72.90 (30)	395.30 ± 14.00 (30)	35.70 ± 7.75 (30)	62.00 ± 0.00 (30)
1	20	25	437.10 ± 26.40 (30)	471.20 ± 23.30 (30)	49.60 ± 15.50 (30)	52.70 ± 9.30 (30)
1	20	40	821.50 ± 37.20 (30)	516.20 ± 29.50 (30)	41.90 ± 12.40 (30)	125.60 ± 20.20 (30)
3.5	20	15	1409.00 ± 76.00 (15)	696.00 ± 49.60 (15)	69.80 ± 12.40 (15)	100.80 ± 10.90 (15)
3.5	20	40	953.30 ± 45.00 (30)	294.50 ± 41.90 (30)	107.00 ± 14.00 (30)	72.90 ± 12.40 (30)
9	20	40	1061.80 ± 37.20 (15)	336.40 ± 18.60 (15)	46.50 ± 12.40 (15)	66.70 ± 12.40 (15)
1	1	100	906.80 ± 65.10 (15)	666.50 ± 45.00 (15)	38.80 ± 7.80 (15)	86.80 ± 18.60 (15)
1	1	333	375.10 ± 14.00 (15)	65.10 ± 15.50 (15)	83.70 ± 23.30 (15)	52.70 ± 9.30 (15)
1	1	500	429.40 ± 37.20 (30)	407.70 ± 23.30 (30)	86.80 ± 9.30 (30)	82.20 ± 6.20 (30)
3.5	1	333	1490.00 ± 48.10 (15)	134.90 ± 23.30 (15)	48.10 ± 12.40 (15)	113.20 ± 24.80 (15)
3.5	1	500	348.80 ± 23.30 (15)	162.80 ± 12.40 (15)	46.50 ± 6.20 (15)	32.60 ± 4.70 (15)

sions were positioned parallel to the border of the mandible 5 mm below the gingival margin. Thus a total of 57 incisions at 19 laser configurations in 19 mandibles were made at this location. Three further incisions were performed in the thicker soft tissues 5 mm from the lower border of the mandible (a total of another 57 incisions in the same 19 mandibles at the same 19 laser configurations at this new, lower location). These two different sites were used to permit identification of gross laser effects in bone underlying the thinner and thicker soft tissues, respectively. Duration of irradiation for each incision measured 4 seconds and was timed with a stopwatch.

Laser device

The laser device (DUOLASE, Medical Optics Inc., Carlsbad, Calif.) emitted at 9.3 μm, the light being delivered by a coherent hollow waveguide and a focusing handpiece. Spot size measured 250 μm. Beam characteristics were calibrated by a laser engineer directly before each irradiation episode, and photographic paper was used to measure and document spot sizes. Beam profiles were single-mode Gaussian. A PRJ-M powermeter (Gentec) was used to determine actual values directly before each laser incision was made.

Laser parameters

The following gated Cw mode parameters were investigated: average power: 1 to 9 W (average power [J/sec] delivered during total duration of irradiation), duty cycle: 10% to 80% (ratio of [laser on time]: [laser on + laser off time]), and pulse duration (length of each individual laser pulse): 1,20,200 msec.

Within 3 minutes of irradiation incisions were dissected out with a margin exceeding 5 mm and were divided into three sections with a fresh, sharp scalpel. This procedure was done because the full-length incisions were too long (3 cm) to fit into the standard paraffin block mountings used for histologic evaluation. Samples were fixed immediately in 10% neutral buffered formalin and were stored in buffered solution under refrigeration until they were embedded in paraffin wax. Wax blocks were prepared, and 6 μm sections were cut according to standard technique and stained with Serius Red. Either 15 or 30 measurements of each category (incision depth, incision width, vertical damage, and horizontal damage) were made per individual incision (Table I). Incision depth and width and depth and width of adjacent tissue damage were measured as depicted in Fig. 2.

In samples where a line of dots resulted from irradiation, measurements were performed centrally within the dot. A photographic record was made of the results.

Statistics

General linear model procedures were performed. All statistical tests were performed at the 0.01 significance level.

RESULTS

Incisional and collateral effects

Mean incision depth and width, mean collateral verti-

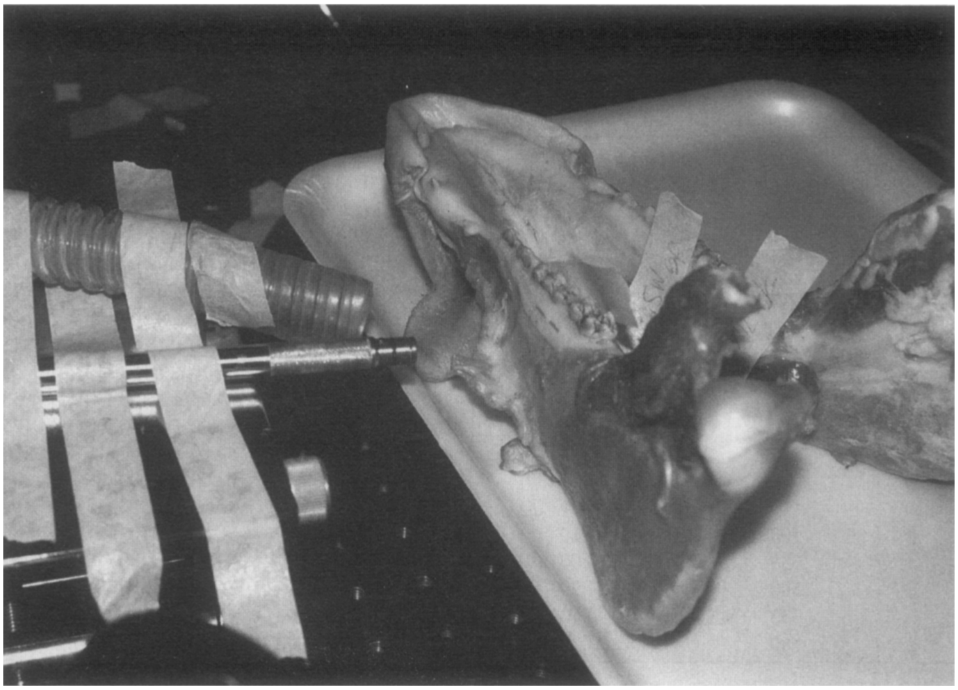

Fig. 1. Experimental configuration.

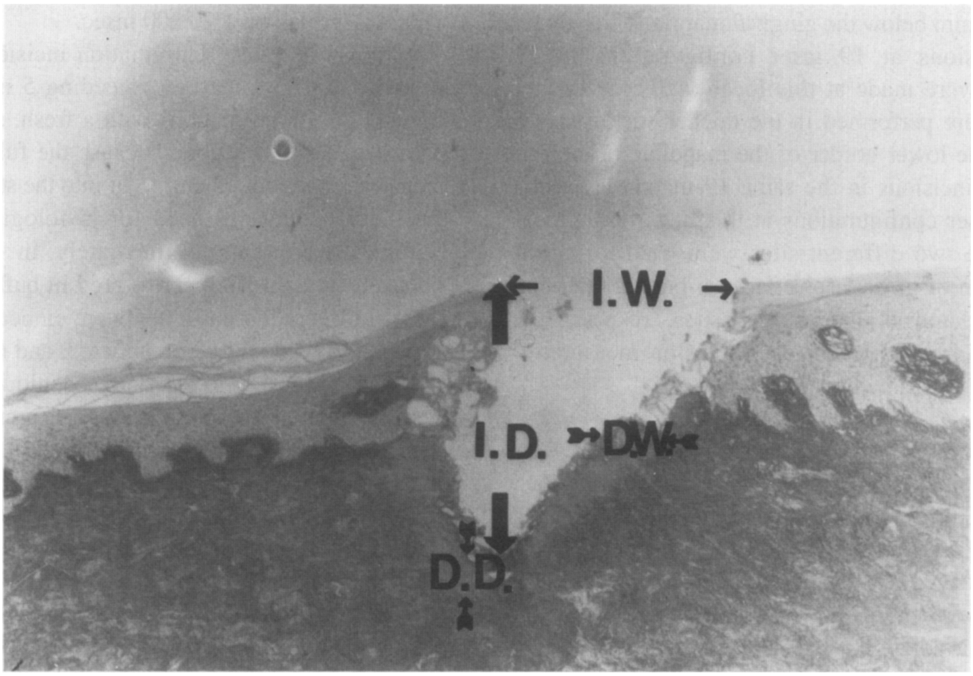

Fig. 2. Measurement sites for histologic evaluation. *ID,* Incision depth; *IW,* incision width; *DD,* tissue damage depth (=vertical damage); *DW,* tissue damage width (=horizontal damage).

cal and horizontal damage, and standard deviations are presented in Table I. The mean incision depth ranged from 327.10 to 1490.00 μm, and the mean incision width ranged from 65.10 to 696.00 μm. The mean vertical damage measured between 34.10 and 107.00 μm, and the mean horizontal damage measured from 32.60 to 153.50 μm. Typical histologic results are presented in Figs. 3 and 4.

Correlation analysis between laser parameters and histologic effects

With the Pearson correlation coefficients, the rela-

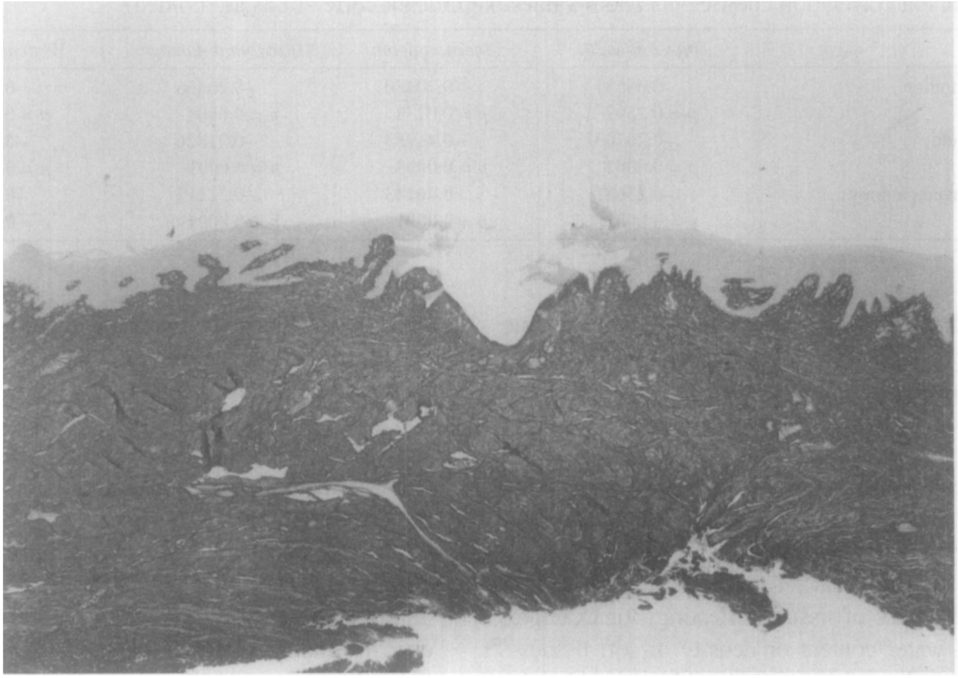

Fig. 3. Typical incision profile at 1 W average power, 1 msec pulse duration, and 500 Hz. Incision is shallow and relatively wide, and collateral damage is moderate.

Fig. 4. Typical incision profile at 9 W average power, 20 msec pulse duration, and 40 Hz. Incision is deep and narrow, and collateral damage is moderate.

tionships depicted in Table II were established. The depth of incision correlated strongly and positively with actual average powers. It also correlated negatively with duty cycle. A highly significant negative correlation existed between incision width and actual average powers and duty cycles. Pulse durations were not significantly correlated with incision depth and width. Horizontal and vertical collateral tissue damages correlated significantly with pulse duration, duty cycle, and average power.

Regression analysis

Regression analyses were used to assess whether pulse duration, duty cycle, or actual average power was predictive of incisional or collateral effects. The regression models considered the main effects of laser parameters and two-factor interactions.

For depth of incision, interactions between duty cycle and actual power ($p < 0.0001$) and between duty cycle and pulse durations ($p < 0.0001$) were identified. The width of incision was associated with a statistically significant interaction between pulse durations and actual power ($p < 0.0009$). Horizontal damage was significantly associated with duty cycle ($p < 0.0380$). For vertical damage interactions between duty cycle and actual power ($p < 0.0021$) and between duty cycle and pulse durations ($p < 0.0002$) were identified.

DISCUSSION

Duty cycle is defined as the temporal ratio between [laser on]:[laser off + laser on]. Because pulsed emissions were spaced linearly per time unit, a higher duty cycle corresponded with a higher pulse repetition rate.

Table II. Pearson correlation coefficients and p values (significant correlations are bolded)

	Incision width	*Incision depth*	*Horizontal damage*	*Vertical damage*
Pulse duration	–0.06531	–0.10480	**–0.20456**	**–0.32514**
	$p = 0.1597$	$p = 0.0238$	**$p = 0.0001$**	**$p = 0.0001$**
Duty cycle	**–0.26703**	**–0.11953**	**–0.31826**	**–0.18469**
	$p = 0.0001$	**$p = 0.0099$**	**$p = 0.0001$**	**$p = 0.0001$**
Actual average power	**–0.23681**	**0.46443**	**–0.32112**	**–0.16053**
	$p = 0.0001$	**$p = 0.0001$**	**$p = 0.0001$**	**$p = 0.0005$**

Because of the different pulse durations used, the same range of duty cycles established throughout this study did not translate into identical pulse repetition rates in the different groupings. For this reason pulse repetition rates are also cited in Table I.

For practical reasons these studies were performed in the mandibles of freshly killed pigs. Use of animal models is common in studies of this sort, yet the reader should keep in mind that laser effects in tissue differ with different types of tissue, reflecting, for example, variations in water content or density of soft tissues.[9] Thus animal studies of this type should be considered a useful indicator rather than a directly transferable predictor of potential laser effects in humans.

Many studies undertaken at 10.6 μm report an average zone of damage after laser incision in soft tissues of < 0.3 mm.[10-14] Thus our results at 9.3 μm fall well within the lower range of these reported histologic effects. However, little information is available on the interrelationship between soft tissue effects and multiple parameter variability at this wavelength.

In evaluating the results of this investigation, a few basic principles of laser-tissue interaction should be kept in mind. The absorption and scattering coefficients of tissues usually change during laser exposure.[8] Therefore the effects of a set configuration of laser parameters on a given tissue will change throughout the course of irradiation. Even minimal carbonization affects further absorption significantly,[8] and scattering of irradiation is already affected at temperatures low enough to cause only coagulation.[8, 15] Dehydration, for example, by the thermal effects of laser irradiation, also greatly alters the optical properties of tissue.[16]

Incision depth tended to decrease with increasing duty cycle. A probable explanation for this observation is that the greater temperature accumulation created by the higher pulse repetition rates creates sufficient surface carbonization and alteration to cause a decrease in the penetration and absorption of irradiation into the underlying tissues. This occurrence is seen, for example, at 200 msec pulse durations and average powers of 1 and 3.5 W. Similar effects were described by Brackett et al.[17] and Halldorsson et al.[18] At 20 msec pulse durations two competing effects were observed: energy penetration was hampered by char formation, countered by the fact that far more energy was being forced into the tissues at the higher pulse repetition rates used. Thus at 1 W average power we measured deepest ablation at 40 Hz (80% duty cycle), least at 25 Hz (50% duty cycle), and intermediate (very close to that of 40 Hz) at 5 Hz (10% duty cycle).

At 1 msec pulse durations pulse repetition rates were far higher than at 20 or 200 msec. At this shorter pulse duration heat transfer effects dominated; the lack of charring and its sequels observed in our samples at this pulse duration are confirmed by Walsh et al.,[19, 20] who reported absence of charring after soft tissue incision at pulse durations < 2 msec. At 1 W average power greater incision depth was observed at the lower pulse repetition rate, despite the fact that fewer pulses and less total energy were being delivered to the tissues. At 3.5 W, 1 msec pulse duration, this effect was even more dramatic (341 μm vs 1488 μm incision depth at 50% and 30% duty cycles, respectively). This finding clearly demonstrates the importance of understanding tissue optical property changes during irradiation over mere consideration of the total energies applied.

Incision depth correlated strongly and positively with average power. This result, confirmed by several other investigations, is logical[19, 21, 22]; deviations from this observation are attributed to the fact that localized heating can build up as a result of absorption centers or inhomogeneities in the tissues, leading to charring and altering entirely the interaction characteristics involved.[8, 17, 18]

Horizontal and vertical damage in tissues adjacent to the incision sites correlated negatively with pulse duration, duty cycle, and average powers. At constant spot size and increasing energy densities achieved by longer pulses, higher pulse repetition rates, or greater powers, an increasing proportion of the irradiated tissues will reach the ablation threshold and become vaporized, leaving smaller residual zones of irradiated and laser-altered tissue behind. These determinants are counterbalanced by the great influence of heat conduction on collateral thermal damage, whereby the width of the damage zone is dependent on heating processes occurring during and after laser cutting.[23] Fitzpatrick et al.[24]

reported a correlation between depth of collateral damage and pulse duration after single pulse firings of 0.05 to 0.5 seconds into soft tissue. In these specimens the consequences of thermal conduction likely exceeded those of ablation after a single relatively long laser pulse. In ablation crater studies on guinea pig skin, Walsh et al.[20] established a correlation between pulse duration and depth of collateral damage with 5 to 50 msec pulse durations without factoring in the effects of pulse repetition rates or power. They also related these observations to heat diffusion processes. Lanzafame et al.[25] determined no significant correlation between pulse repetition rates, power, and histologic effects with the CO_2 laser in the chop-wave mode at far higher pulse repetition rates (300 to 1000 Hz) and powers (25 W average power) and generated greater and more variable zones of collateral damage than those observed in our investigation. All of the parameters used in those investigations will have produced relatively powerful thermal effects, overshadowing the influence of other determinants.

In summary, this study investigated events resulting from soft tissue incision with a CO_2 laser emitting at 9.3 µm. Incisional and histologic effects were related to parameters used but were multifactorially governed. The depth of incision correlated strongly and positively with average power and negatively with duty cycle. Tissue damage correlated strongly and negatively with all three variables: average power, duty cycle, and pulse duration. These results are clinically significant because they demonstrate that a wide range of surgical effects can be achieved with one specific laser device depending on the parameter configuration selected. Moreover, laser parameters must be selected carefully to comply with thermal limitations in target and collateral tissues.

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Reprint requests:
Petra Wilder-Smith, Dr. Med. Dent., BDS (Hons.) LDSRCS (Eng)
Director of Dentistry and Assistant Professor
Beckman Laser Institute and Medical Clinic
1002 Health Science Rd. East
University of California, Irvine
Irvine, CA 91715