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ORIGINAL ARTICLE

Reduction of collateral thermal impact of diode laser irradiation on soft tissue due to modified application parameters

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Abstract The aim of this study was 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. A total of 108 bovine liver samples were cut with a diode laser at various settings in pulsed and micropulsed mode and histologically assessed to determine the area and depth of carbonization, necrosis and reversible tissue damage, as well as incision depth and width. Incision depth and width and the area and depth of carbonization, necrosis and reversible damage were correlated strongly with cutting speed. The area and depth of reversible damage were correlated with average power. The micropulsed mode produced a smaller zone of carbonization and necrosis and a smaller incision width. Setting the laser parameters in accordance with the absorption characteristics of the tissue reduced collateral thermal tissue damage while maintaining an acceptable cutting ability. Reducing collateral thermal damage from diode laser incisions is clinically relevant for promoting wound healing.

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Introduction

The scalpel and the electrosurgery unit (ES) are the instruments of choice for soft-tissue surgery especially concerning accuracy, speed and cost. In contrast, lasers are a more innovative approach providing a clear almost bloodless operative field combined with excellent cutting ability. Surgical interventions including those involving the lips, attached and unattached mucosa and the free gingival margins (for example, frenectomies, gingival excisions, peri-implant soft-tissue surgery and removal of soft-tissue tumours) are very common. After laser surgery a lack of swelling, bleeding, pain and scar tissue formation are seen in a high percentage of patients [1]. Stübinger et al. reported the use of the diode laser in 40 patients and found less intra- and postoperative pain and no wound healing disorders [2].

However, cutting soft tissue with a laser always produces a certain amount of thermal damage horizontally and vertically around the incision as shown by an area of carbonization, necrosis and reversibly altered tissue (tissue that shows thermally induced changes which heals without any defect). This tissue damage may result in impeded wound healing compared with healing of a scalpel incision [3]. The absorption maximum of the diode laser (980 nm) correlates with the absorption range of haemoglobin, thus suggesting that liver tissue would show a considerable effect while cutting. However, cutting soft tissue with a laser is considered to be primarily 100% sterile [4].

In contrast, others have reported that the horizontal and vertical damage zone around diode laser incisions depends neither on the average power, nor on the mode used or the dimension of the fibre tip. There is one characteristic difference between the carbon dioxide laser and the diode laser, that is with the latter laser there is no trend towards greater lateral damage to tissues with continuous wave mode at higher power levels [5]. We emphasize that the characteristics of pulsed lasers (carbon dioxide, Nd:YAG) make them unsuitable for diode lasers as the latter operate in continuous or chopped mode only. It is a persisting incongruity in nomenclature that the diode laser works in "pulsed mode". Maximum output is always equal to the nominal output of the device. Nevertheless, for the sake of concordance and simplicity we stick to the, albeit wrong, nomenclature.

Studying differences in wound healing sequences between the three devices (scalpel, ES and laser), it has been found that inflammation and reepithelialization are best in scalpel wounds after 3 days but comparable among all the devices after 1 week. However, after 6 weeks less granulation tissue formation was found in scalpel and laser wounds than in ES wounds [6]. These results confirm the findings of Tipton et al. that scalpel wounds show higher tensile strength and much faster healing than ES wounds [7].

Reducing the area of collateral thermal tissue damage to a minimum while maintaining cutting ability is the major objective of current research to minimize the risk of impaired wound healing.

Materials and methods

Three pieces of capsular bovine liver were used in this study. The organs were cooled to 4°C immediately after the animal was killed and within 24 h returned to room temperature before preparation. The temperature was checked with a standardized penetration thermometer (BT 20/DT 131; Trotec, Heinsberg, Germany). Twelve square pieces were taken from the major lobe of each liver to give a total of 36 lobules. A diode laser (GENTLEray; KaVo Dental, Biberach, Germany) emitting at a wavelength of 980 nm was used in pulsed and micropulsed mode with 2.5, 3.5 and 4.5 W displayed. In pulsed mode these settings produced peak powers of 2.5, 3.5 and 4.5 W giving average powers of 1.2, 1.8 and 2.2 W, respectively. In micropulsed mode these settings produced a peak power of 12 W at average powers of 1.2, 1.8 and 2.2 W. The incisions were made with a 300-µm fibre tip in contact. To ensure maximum comparability and a highly standardized procedure for the 108 samples, a controlled, scientifically proven test assembly, moving the hand-piece automatically, was used to reduce subjective effects on the laser application to a minimum [8].

After preparation and confirmation of the temperature, the square tissue lobules were pinned onto a moveable computer-

ized table. Two runs with the same laser parameters were carried out on each lobule. A run included three incisions, 24 mm in length made in 24, 48 and 72 s. The speed and length of the incisions and their spacing were standardized, controlled and recorded in a protocol by the associated computer. Immediately after laser irradiation each tissue sample was fixed in 4% neutral-buffered formaldehyde and temporarily stored in an air-tight container. A total of 108 wax blocks were prepared, and 3- μ m sections were cut with a rotary microtome (Accu-Cut SRM 200; Sakura Finetek, Torrance, CA) and stained with Martius scarlet blue.

Histomorphometric analysis was performed with special purpose-designed software (Definiens Developer XP) which calculated the microscopic image of each slide (Fig. 1). The results of the software calculations are shown in Fig. 2. The software drew the lines between the areas in accordance with their different coloration, and in addition every slide was checked by a cell biologist to modify the lines in accordance with the morphology. After the lines were set the data could be calculated.

Our research was carried out with the consent of and at the Bernhard Gottlieb Dental School, Medical University of Vienna. As we used regular abattoir material, approval from the Ethics Commission was not required in accordance with Austrian law.

Results

Mean incision width and depth, and mean area and depth of carbonization, necrosis and reversible damage are shown in Tables 1 and 2.

Analysis of covariance showed that all eight parameters were correlated strongly with cutting speed. There were no correlations between average power and incision depth and width, area and depth of carbonization and necrosis. Only



Fig. 1 Microscopic image of a histological slide



Fig. 2 Software-calculated zones of thermal damage (*black* area of carbonization, *red* area of necrosis, *pink* area of reversible damage)

the area and depth of reversible damage were correlated with average power, as shown in Tables 3 and 4.

Pulsed mode was associated with a larger zone of carbonization, and area and depth of necrosis as well as a greater incision width than micropulsed mode.

Discussion

Diode lasers are very common and their practical use in head and neck surgery is well documented. In contrast to this more clinical approach, there are only a few in vitro evaluations of the histological side effects of different wavelengths according to the tissue [5, 6]. (However, wavelengths of 600–1,000 nm are most commonly used in pulsed or micropulsed mode. This is documented in a lot of papers on the clinical applications [1, 2].) The use of diode lasers is well known in liver surgery [9] and for focal

laser-induced hyperthermia therapy against liver tumours and metastasis [10, 11]. In surgical indications (for example, in liver surgery) it has been proven that diode lasers produce a quite acceptable collateral damage zone [12].

The application of diode laser energy (805 nm, average power 3.6 W) to liver tissue results in complete cell membrane and nuclear disruption in the first four or five cell lavers (50 µm) [9]. The depth of the induced area of necrosis depends on the optical and physiological properties of the treated tissue [13]. In evaluating wavelengths it is important to remember that the laser effects on soft tissue are directly related to the light absorption characteristics of the tissues, and to the laser parameters used. For example, Carbon dioxide lasers (with a wavelength at about 10 µm) have a high absorption coefficient in water and collagen, and therefore usually produce narrower zones of tissue damage in soft tissues than the Nd:YAG laser [14, 15]. Evaluating data for the near-infrared spectrum, Matcher et al. found several absorption coefficients from, for example, haemoglobin, water and fat [16, 17]. For haemoglobin there is a slight increase in absorption at a wavelength of 980 nm, but maxima are set between 500and 600 nm [16-20]. This means that the diode laser does not perfectly match single components but overall offers a quite suitable medium.

In this study we chose liver tissue as it matches the optical properties better than dead bloodless epithelium while still showing enough similarity to oral tissue. Furthermore, it is much easier to avoid subjective influences by pinning the flat soft tissue samples to a computerized table. Heat accumulation is one of the most important factors [13, 21]. The relationship between cumulative energy deposition and the lasing protocol (laser duration and tissue target temperature) is considered to be linear with higher energy deposition with longer lasing times and higher tissue temperatures [11]. Haemoglobin as the absorbing constituent of the tissue used

Table 1 Dimensions of laser incisions and collateral effects of the laser in micropulsed mode (values are means \pm SD, n=54)

Average power (W)	Speed (mm/s)	Area of carbonization (mm ²)	Depth of carbonization (mm)	Area of necrosis (mm ²)	Depth of necrosis (µm)	Area of reversible damage (mm ²)	Depth of reversible damage (mm)	Incision width (mm)	Incision depth (mm)
2.5	0.33	$0.020 {\pm} 0.008$	0.020 ± 0.006	0.281±0.082	0.138±0.024	1.272±0.314	$0.442 {\pm} 0.084$	0.472±0.217	1.202±0.172
2.5	0.5	0.015 ± 0.016	$0.017 {\pm} 0.008$	$0.183 {\pm} 0.083$	$0.108 {\pm} 0.015$	0.811 ± 0.229	$0.349 {\pm} 0.061$	$0.427 {\pm} 0.272$	1.103 ± 0.299
2.5	1	0.007 ± 0.005	$0.012 {\pm} 0.003$	$0.071 {\pm} 0.029$	$0.067 {\pm} 0.012$	$0.297 {\pm} 0.085$	$0.197 {\pm} 0.035$	$0.176 {\pm} 0.090$	0.865±0.167
3.5	0.33	0.027 ± 0.019	0.020 ± 0.005	$0.333 {\pm} 0.106$	$0.136 {\pm} 0.019$	$1.799 {\pm} 0.389$	$0.512 {\pm} 0.104$	$0.636 {\pm} 0.349$	1.216±0.422
3.5	0.5	0.013 ± 0.009	0.013 ± 0.007	$0.181 {\pm} 0.100$	$0.098 {\pm} 0.043$	$0.789 {\pm} 0.268$	$0.350 {\pm} 0.044$	$0.448 {\pm} 0.284$	0.950 ± 0.595
3.5	1	0.002 ± 0.001	$0.008 {\pm} 0.004$	$0.070 {\pm} 0.019$	$0.068 {\pm} 0.012$	0.241 ± 0.062	$0.167 {\pm} 0.048$	$0.177 {\pm} 0.120$	0.689 ± 0.375
4.5	0.33	$0.059 {\pm} 0.036$	0.030 ± 0.012	$0.379 {\pm} 0.106$	$0.147 {\pm} 0.035$	2.274±1.109	$0.607 {\pm} 0.227$	$0.753 {\pm} 0.238$	1.298±0.106
4.5	0.5	$0.018 {\pm} 0.009$	$0.020 {\pm} 0.007$	$0.156 {\pm} 0.069$	0.101 ± 0.036	1.098 ± 0.335	$0.472 {\pm} 0.081$	0.313 ± 0.116	$1.018 {\pm} 0.078$
4.5	1	$0.011 {\pm} 0.014$	$0.014 {\pm} 0.010$	$0.105 {\pm} 0.070$	$0.077 {\pm} 0.024$	$0.552 {\pm} 0.280$	$0.301 \!\pm\! 0.080$	$0.308 {\pm} 0.163$	0.801 ± 0.447

Average power (W)	Speed (mm/s)	Area of carbonization (mm ²)	Depth of carbonization (mm)	Area of necrosis (mm ²)	Depth of necrosis (mm)	Area of reversible damage (mm ²)	Depth of reversible damage (mm)	Incision width (mm)	Incision depth (mm)
2.5	0.33	0.025±0.019	0.023 ± 0.006	0.267±0.087	0.137±0.017	1.107±0.221	$0.435 {\pm} 0.043$	0.366±0.159	1.291±0.191
2.5	0.5	$0.018 {\pm} 0.015$	$0.020 {\pm} 0.007$	$0.190 {\pm} 0.047$	$0.117 {\pm} 0.018$	$0.638 {\pm} 0.177$	$0.316 {\pm} 0.045$	$0.327 {\pm} 0.114$	1.121 ± 0.173
2.5	1	$0.017 {\pm} 0.019$	$0.017 {\pm} 0.010$	$0.106 {\pm} 0.057$	$0.079 {\pm} 0.025$	$0.285 {\pm} 0.093$	0.193 ± 0.033	$0.233 {\pm} 0.173$	$0.849 {\pm} 0.429$
3.5	0.33	$0.037 {\pm} 0.015$	$0.029 {\pm} 0.011$	0.473 ± 0.107	$0.190 {\pm} 0.025$	1.762 ± 0.546	$0.502 {\pm} 0.098$	$0.462 {\pm} 0.200$	$1.477 {\pm} 0.258$
3.5	0.5	$0.040 {\pm} 0.032$	$0.026 {\pm} 0.011$	$0.379 {\pm} 0.148$	$0.161 {\pm} 0.031$	$1.278 {\pm} 0.350$	$0.435 {\pm} 0.068$	$0.555 {\pm} 0.263$	1.449 ± 0.209
3.5	1	$0.016 {\pm} 0.005$	$0.018 {\pm} 0.003$	$0.149 {\pm} 0.029$	$0.099 {\pm} 0.014$	$0.497 {\pm} 0.155$	$0.256 {\pm} 0.062$	$0.338 {\pm} 0.215$	$0.934 {\pm} 0.206$
4.5	0.33	$0.047 {\pm} 0.020$	$0.032 {\pm} 0.012$	$0.492 {\pm} 0.165$	$0.160 {\pm} 0.028$	2.102 ± 0.547	$0.528 {\pm} 0.123$	$0.838 {\pm} 0.397$	$1.737 {\pm} 0.418$
4.5	0.5	$0.024 {\pm} 0.008$	$0.021 \!\pm\! 0.005$	$0.289 {\pm} 0.109$	$0.124 {\pm} 0.024$	$1.142 {\pm} 0.232$	$0.385 {\pm} 0.064$	$0.657 {\pm} 0.407$	$1.336 {\pm} 0.327$
4.5	1	$0.016 {\pm} 0.008$	$0.020 {\pm} 0.004$	$0.116 {\pm} 0.017$	$0.077 {\pm} 0.009$	$0.563 \!\pm\! 0.059$	$0.254{\pm}0.037$	$0.377 {\pm} 0.183$	$1.138 {\pm} 0.303$

Table 2 Dimensions of laser incisions and collateral effects of the laser in pulsed mode (values are means \pm SD, n=54)

in this study has been shown to be able to take up oxygen for up to 48 h after death; cooling the tissue between death and the use of the tissue enhances this ability [22].

The histological effects of the carbon dioxide laser have been very well evaluated. For example, histologically evident effects of the carbon dioxide laser have been reported to extend approximately 60 µm into soft tissues [23]. These findings have been confirmed by those of Wilder-Smith et al. that fell well within the range of previously reported histological effects [24]. Although it is true that 99% of laser light is absorbed within 60 µm, the use of high power densities in continuous wave mode can lead to accumulation of heat and thermal damage to adjacent structures. The zones of vaporization and damage from heat conduction will depend directly on the laser parameters [24, 25]. This finding is directly relevant to clinical dentistry because of the closeness of teeth and bone in the oral cavity. The use of the carbon dioxide laser in superpulsed mode, based on the principles of high irradiance with short pulse duration and adequate pulse intervals, has been shown to reduce thermal necrosis by a factor of two or more [26, 27].

eversible damage

Versus	Value \pm SD (mm ²)	<i>p</i> -value
Pulsed vs. micropulsed	$0.0851 {\pm} 0.0747$	0.2723
3.5 vs. 2.5 W	$0.1452 {\pm} 0.0457$	0.0063*
4.5 vs. 3.5 W	$0.1452 {\pm} 0.0457$	0.0063*
0.33 vs. 0.5 mm/s	$0.7598 {\pm} 0.1027$	< 0.0001*
0.33 vs. 1 mm/s	1.3135±0.0955	<0.0001*
0.5 vs. 1 mm/s	$0.5537 {\pm} 0.0419$	<0.0001*

*p<0.05

Still, characteristics of pulsed lasers (carbon dioxide, Nd: YAG) make them unsuitable for diode lasers as the latter operate in continuous or chopped mode only. As mentioned above, it is a persisting incongruity in nomenclature that the diode laser works in "pulsed mode". Maximum output is always equal to the nominal output of the device. Nevertheless, for the sake of concordance and simplicity we stick to the, albeit wrong, nomenclature.

As the diode laser represents a good compromise for daily use in dental offices, finding use in many dental indications (e.g. surgery, periodontology, endodontology), minimization of collateral damage is important to improve the therapeutic outcome. This study clearly showed that micropulsed mode—maximum energy with the largest pause-to-pulse ratio—can minimize the collateral thermal damage in soft tissue. Furthermore, this reduction in collateral thermal damage during the application of micropulsed mode is not associated with a reduction in the cutting ability of the diode laser. These findings are particularly important for clinical use of the laser because necrotic tissue directly impairs wound healing.

Table 4 Depth of reversible damage

Versus	Value \pm SD (mm)	<i>p</i> -value
Pulsed vs. micropulsed	-0.0017 ± 0.0262	0.9492
3.5 vs. 2.5 W	0.0462 ± 0.0160	0.0114*
4.5 vs. 3.5 W	0.0462 ± 0.0160	0.0114*
0.33 vs. 0.5 mm/s	$0.1200 {\pm} 0.0183$	< 0.0001*
0.33 vs. 1 mm/s	$0.2766 {\pm} 0.0181$	< 0.0001*
0.5 vs. 1 mm/s	$0.1565 {\pm} 0.0105$	<0.0001*

*p<0.05

Conclusion

The use of the right laser parameter settings in accordance with the absorption characteristics of the tissue enables collateral thermal tissue damage to be reduced while maintaining proper cutting ability. Further studies are needed to clarify if the reduced collateral damage shown in this study with the use of micropulsed mode can lead to accelerated wound healing. It remains to be seen if an additional cooling system could further improve the cutting ability of the diode laser together with a further reduction in thermal damage.

Conflicts of interest The authors declare that they have no conflicts of interest.

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