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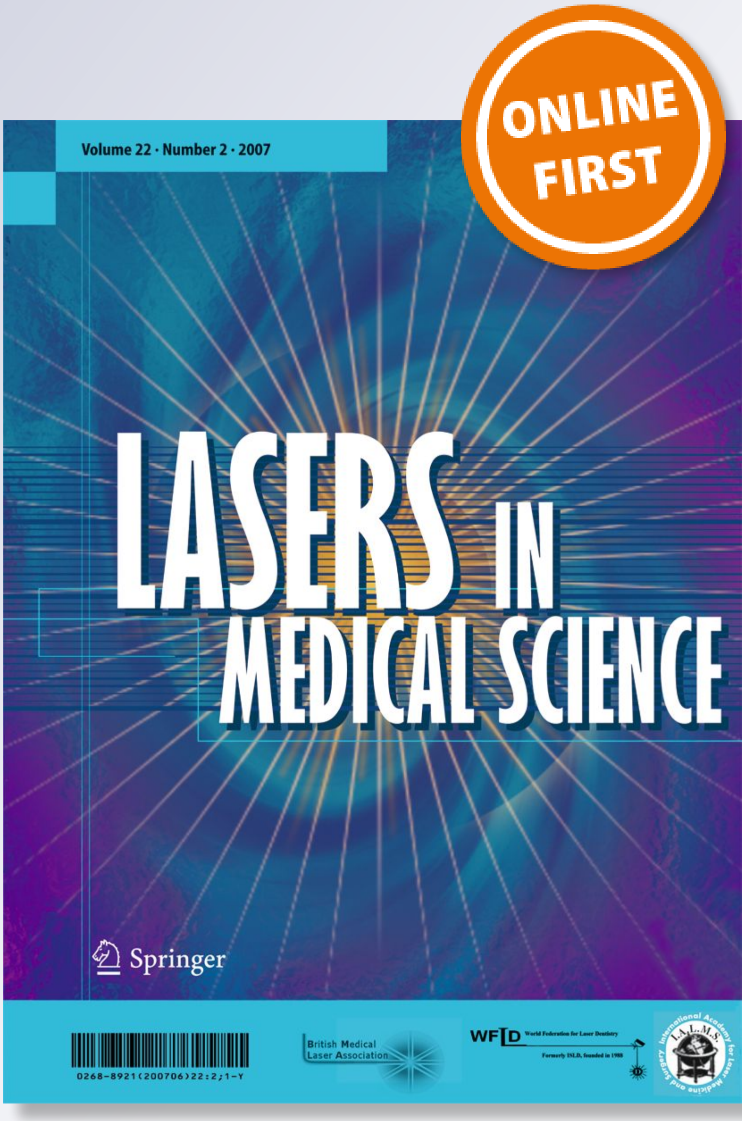

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ORIGINAL ARTICLE

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

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Abstract Since the diode laser is a good compromise for the daily use in dental offices, finding usage in numerous dental indications (e.g., surgery, periodontics, and endodontics), the minimization of the collateral damage in laser surgery is important to improve the therapeutical outcome. The aim of this study was to investigate the effect of water/air cooling on the collateral thermal soft tissue damage of 980-nm diode laser incisions. A total of 36 mechanically executed laser cuts in pork liver were made with a 980-nm diode laser in micropulsed mode with three different settings of water/air cooling and examined by histological assessment to determine the area and size of carbonization, necrosis, and reversible tissue damage as well as incision depth and width. In our study, clearly the incision depth increased significantly under water/air cooling (270.9 versus 502.3 μm—test group 3) without significant changes of incision width. In test group 2, the total area of damage was significantly smaller than in the control group (in this group, the incision depth increases by 65 %). In test group 3, the total area of damage was significantly higher (incision depth increased by 85 %), but the bigger part of it represented a reversible tissue alteration leaving the amount of irreversible damage almost the same as in the control group. This first pilot study clearly shows that water/air cooling in vitro has an effect on collateral tissue damage. Further studies will have to verify, if the reduced collateral damage we have proved in this study can lead to accelerated wound healing. Reduction of collateral thermal damage after diode laser incisions is clinically relevant for promoted wound healing.

Keywords Diode laser · Thermal damage · Micropulsed · Soft tissue · Surgery

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Introduction

The efficiency of the laser–tissue interaction depends mainly on the wavelength, energy, operation mode, and the optical properties of the target tissue [1]. High absorption of the tissue leads to evaporation and cutting abilities whereas low absorption only leads to coagulation and heat application [2]. CO_2 lasers for example (with a wavelength of 10.600 nm) 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 [3, 4].

When laser light strikes the tissue, it will always produce a certain amount of thermal damage (carbonization, necrosis, and reversible damage) encircling the intended incision depth and width, thus resulting in a greater tissue damage which may cause impeded wound healing when compared

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with a scalpel incision [5]. For example, it is well documented in literature that wound healing sequence after 1 week is best in scalpel wounds, yet comparable to laser wounds after 6 weeks [6]. The highest rate of granulation tissue formation was found in electrosurgery wounds. The reason for this delay in wound healing is the necrotic tissue that needs to be degraded and replaced by physiological repair mechanisms [5, 7]. Therefore, minimization of the collateral damage in laser surgery is important to improve the therapeutical outcome.

In order to reduce the unwanted heat accumulation, there have been attempts of combining the laser device with a cooling system. Such cooling systems for example have been developed for the CO_2 the Er, Cr/YSGG, or the Er/YAG laser [8–11].

According to nowadays' literature, the diode laser proves to be a good compromise in dental offices, being applied in numerous indications (e.g., periodontics, endodontics, bleaching, or low-level laser therapy) [12–15]. Using the diode laser in surgical indications reports certain advantages as well (like providing a clear, almost bloodless operation field and satisfying cutting abilities combined with post surgical lack of swelling, bleeding, pain, or scar tissue), but no cooling system has been developed so far [16].

In today's laser surgery, the reduction of collateral thermal tissue damage area to a minimum while maintaining proper cutting abilities are the major objectives in minimizing the risk of impaired wound healing.

What is even more important is to emphasize that characteristics of pulsed lasers (e.g., CO_2 and Er/YAG) are not suitable for diode lasers, as they operate in continuous, chopped, or gated-pulsed ("micropulsed") mode only. It is a persisting incongruity in nomenclature that the diode lasers work in "pulsed or micropulsed mode." A diode laser's "micropulsed" mode is an intermittent sequence of pulse complexes, and the maximum output is always equal to the nominated output of the device. Nevertheless, in terms of accordance and simplicity, we stick to this expression, albeit wrong nomenclature.

The aim of this study was to investigate the effect of water/air cooling on the collateral thermal soft tissue damage of 980-nm diode laser incisions

Material and methods

Test assembly

For the purpose of this investigation, four pieces of capsular pork liver were utilized. The organs were kept at a temperature of 5 °C immediately after explantation and within 24 h brought back to room temperature before preparation. Temperature was checked with a standardized penetration thermometer (Trotec® BT 20/DT 131; Trotec GmbH, Heinsberg Germany). Three square pieces from the major lobe of each liver (*lobus dexter lateralis et medialis*) were taken and separated in three different test groups (TG 1, TG 2, and TG 3).

To assure maximum comparability and a highly standardized conduct of the sampling procedure through the 36 samples, a controlled, scientifically proven test assembly, moving the hand piece automatically, was chosen to reduce individual influence on the laser application to a minimum. Investigations on this device showed that the precision of the guiding instrument for laser cutting reduces the error of cut width by 50-fold. In this way, comparative laser studies can be accomplished objectively [17].

A diode laser (GENTLEray, KaVo Dental GmbH, Biberach, Germany) emitting at a wavelength of 980 nm was used in micropulsed mode with 3 watt (W) displayed. This means a peak power of 12 W giving an average power of 1.5 W. The incisions were made in contact with a 300-μm fiber tip.

Since there is no proper cooling system for diode lasers available so far, water/air cooling was provided by an Er, Cr/YSGG laser (Waterlase MD™, Biolase Technology, Inc., Irvine, CA) which was experimentally attached to the diode laser and emitted a water spray at three different settings (Table 1).

Test group 1: 5 % water and 50 % air
Test group 2: 5 % water and 75 % air
Test group 3: 5 % water and 100 % air

After preparation and temperature affirmation, the square tissue lobules (4×4.5 cm and about 0.8 cm thick, measured with a one-way paper ruler used for histological examinations) were pinned onto the moveable, computerized table. Two runs with the same laser settings and cooling parameters and a control without cooling were carried out on each lobule. The three incisions with a length of 24 mm with a distance of 10 mm from each other were made in 72 s, which is approximately 0.33 mm/s. Speed, length, and space between the incisions were standardized, controlled, and recorded in a protocol by the associated computer.

Immediately after laser irradiation, each tissue sample was marked by a surgical suture on the left lower side and fixed in 4 % neutral-buffered formaldehyde.

Histological examination

All storage boxes were closed air tight and cooled to 4 °C (39.2 °F). A week later, all tissue lobules were washed, labeled and, according to the three laser incisions, cut into three pieces.

A 3- to 4-mm block from each laser cut was taken and stored in 70 % isopropanol. After embedding in paraffin (Tissue-Tek® VIP™; Sakura® Finetek USA, Inc., Torrance, CA), all specimen were consecutively covered with liquid wax, formed to blocks, and cooled to −5 °C (23 °F). A total

Table 1 Test groups and applied air/water cooling (TG)

TG 1	1st lobe liver #1	2 cuts with 5 % water and 50 % air—1 cut without cooling
	1st lobe liver #2	2 cuts with 5 % water and 50 % air—1 cut without cooling
	1st lobe liver #3	2 cuts with 5 % water and 50 % air—1 cut without cooling
TG 2	2nd lobe liver #1	2 cuts with 5 % water and 75 % air—1 cut without cooling
	2nd lobe liver #2	2 cuts with 5 % water and 75 % air—1 cut without cooling
	2nd lobe liver #3	2 cuts with 5 % water and 75 % air—1 cut without cooling
TG 3	3rd lobe liver #1	2 cuts with 5 % water and 100 % air—1 cut without cooling
	3rd lobe liver #2	2 cuts with 5 % water and 100 % air—1 cut without cooling
	3rd lobe liver #3	2 cuts with 5 % water and 100 % air—1 cut without cooling

of 36 wax blocks was prepared and 3 μm sections cut with a microtome (Accu-Cut SRM™ 200 Rotary Microtom; Sakura® Finetek USA, Inc., Torrance, CA).

After the histological specimens were attached to the panels they were stained with Martius Scarlet Blue using several coloration series according to the attached protocol and rapidly dehydrated (Tissue-Tek® DRS™ Fa. Sakura Finetek USA, Inc., Torrance, CA).

Once the coloration was finished, the specimens were provided with a cover glass and temporarily stored in a proper box until histomorphometric analysis.

Histomorphometric analysis was conducted with a special purpose-built software (Definiens Developer XP®) which calculated the microscopic pictures of each slide. The principle of histomorphometric analysis is shown in Fig. 1.

The pictures show the laser cut with a certain depth and width and the different areas of destruction marked with different colors. "Black" is the area of carbonization, "dark red" is the area of necrosis and "light red" is the zone of reversible damage. Black and dark red can be combined to the area of irreversible damage. All data were taken in pixel—1 pixel= 1.12736 μm.

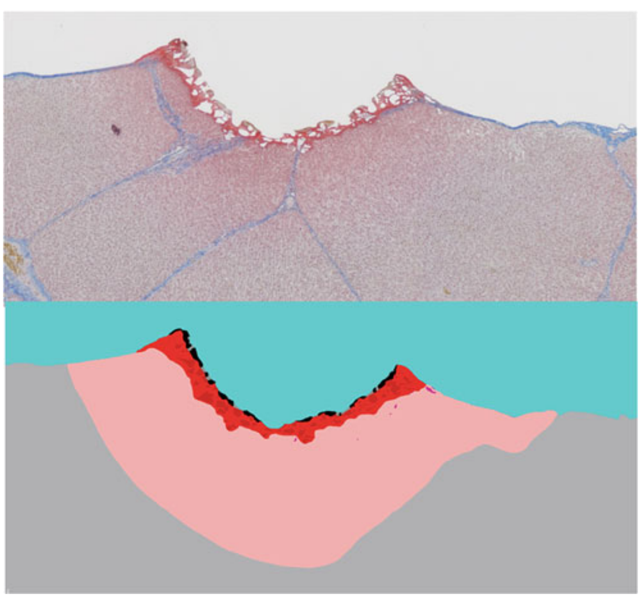

Fig. 1 Microscopic and histomorphometric picture of the histological slide. The area of carbonization is *colored black*, area of necrosis *red*, and the area of reversible damage is *colored pink*

Statistical analysis

Descriptive data analysis was performed by calculating the mean value, standard deviation, and a 95 % confidence interval which is shown in the squared brackets.

Analytical data evaluations were carried out by calculation of absolute and relative frequency. Comparisons between the groups were drawn by a "*t* test" (water or no water) and by a multivariate analysis of variance (water/air cooling with the three different ratios). After that a linear contrast was calculated between the different groups—"Scheffé-test."

Results

After cutting liver tissue with a diode laser, the histological picture shows three different zones of damage from the border of the laser cut to the exteriors [18]. The zone of carbonization—tissue is completely destructed, the water evaporated, and a black layer remains covering the residual tissue. The zone of necrosis—tissue is also devital, cell membranes are damaged, proteins and enzymes denatured, but the histological composition of the harmed tissue is still detectable. The zones of carbonization and necrosis are also called the zone of irreversible damage. Vital tissue is found underneath these two layers. The zone of reversible damage—cells are slightly impaired but can recover due to physiological repair mechanisms. Consecutively, the physiological tissue morphology reappears [19].

The main question of the statistical analysis was to investigate if it is possible to reduce the zone of irreversible tissue damage (which actually slows down wound healing) by water/cooling while maintaining proper incision efficiency of the laser.

Incision efficiency

All test groups showed increased cutting depth without statistically significant differences in cutting width (Table 2; Fig. 2). The amount increases from test groups 1 to 2 and 3. In the last group, the cutting depth even increased by 85 % compared with the control group.

Tissue damage analysis

"Tissue damage" was defined as the whole area of tissue alteration or just the thickness of layers beneath the cut. Mean thickness, mean area, and number of pores in the three zones of damage (carbonization, necrosis, and reversible damage) are shown in Table 3.

Thickness Data analysis demonstrated an almost equal value of total damage in control and test group 2 with a significant higher proportion of carbonization in the test group 2. The test groups 1 and 3 showed a higher amount of reversible damage without differences in the area of irreversible damage. Referring to the proportion of irreversible damage to the total thickness of damage, only test group 2 statistically increased (Fig. 3).

Area Specific data evaluations of area of the three damage zones are similar to the values of thickness but are most relevant. The total amount of damage is lowest in test group 2. The highest increase of total area of damage is measured in test group 3 but without significant increase of irreversible damage. According to the proportion of irreversible damage to the total area of damage, only test group 2 is statistically increased (Fig. 4; Table 4).

Pores In terms of the ration of the number of pores between the test groups and the control group, a significantly higher value in the zone of carbonization could be measured in the test groups.

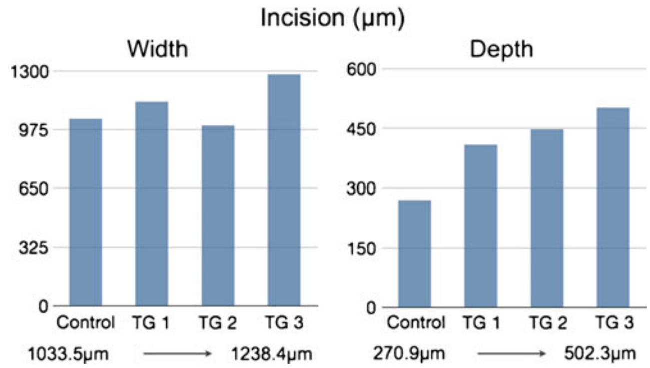

Fig. 2 Incision depth and width

Discussion

Evaluations of earlier studies about diode laser incisions and wound repair are quite disputable because most of them were made free hand and therefore implicate a personal bias in handling of the device [5, 7, 16, 18, 20]. That is why we eliminate individual influence completely by choosing a fully automated incision system [17].

On the other hand, in early studies particularly, diode laser incisions, when compared with scalpel, always showed an increased inflammation during wound healing resulting from the necrotic tissue that needs to be removed and replaced by the body [5, 7].

Therefore, setting up therapeutic goals leads to a proportional change of the damaged tissue towards a quantitative decrease of irreversible and an increase of reversible damage.

One way of minimizing collateral thermal damage is maximum absorption by optimizing the correlation of the laser wavelength and the absorption characteristics of the target tissue.

CO_2 lasers for example 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 [3, 4].

Histologically evident effects of the CO_2 laser extend approximately 60 µm into soft tissue [21]. Authors have demonstrated that the use of a super pulsed mode in CO_2 lasers, based on the principles of high irradiance with short pulse duration and adequate pulse intervals will reduce thermal necrosis by a factor of 2 or more [22, 23]. Er/YAG laser have a high absorption coefficient in water [24].

According to the diode laser, there is an increase in the absorption coefficient of hemoglobin at 980 nm, but maxima are set between 500 and 600 nm [25–29]. However, Angiero et al. proved that the diode laser is a valid therapeutic instrument for excising oral lesions larger than 3 mm in diameter [30].

Table 2 Incision depth and width

Incision (µm)	Control (mean (CI))	Cooling 1 (mean (CI))	Cooling 2 (mean (CI))	Cooling 3 (mean (CI))	*p* value
Width	1,033.49 (872.26; 1,194.73)	1,127.87 (985.17; 1,270.57)	997.41 (820.04; 1,174.78)	1,283.42 (1,040.80; 1,526.05)	0.084
Depth	270.88 (173.29; 368.47)	407.55 (281.45; 533.64)	447.83 (301.56; 594.12)	502.33 (348.96; 655.80)	0.022

CI confidence interval

Table 3 Thickness, area, and number of pores in the three zones of damage

	Control group (mean ± SD (CI))	Test group 1 (mean ± SD (CI))	Test group 2 (mean ± SD (CI))	Test group 3 (mean ± SD (CI))
Carbonization				
Thickness (µm)	31.95±11.48 (24.66; 39.24)	30.51±10.10 (22.06; 38.95)	50.73±21.30 (32.93; 68.53)	37.30±12.15 (27.15; 47.46)
Area (µm^2)	32,009.51±15.665.74 (22,055.98; 41,963.04)	35,699.53±15,227.61 (22,938.92; 48,400.14)	54,628.77±33.542.62 (26,586.43; 82,671.10)	52,568.66±18,533.37 (37,074.37; 68,062.94)
Pores (*n*)	374.33±98.90 (311.50; 437.17)	438.25±110.45 (345.91; 530.59)	450.75±126.27 (345.18; 556.32)	546.38±111.52 (453.15; 639.60)
Necrosis				
Thickness (µm)	90.46±11.24 (63.31; 97.60)	97.59±19.93 (80.93; 121.37)	92.99±16.82 (78.93; 107.05)	84.50±16.50 (70.71; 98.29)
Area (µm^2)	84,507.38±22,245.41 (70.373.32; 98,641.43)	93,545.66±30,968.70 (67,355.17; 119,436.14)	103,071.00±44,573.42 (65,806.69;140,335.32)	94,726.14±19,134.98 (78,728.89; 110,723.39)
Pores (*n*)	210.17±108.09 (141.49; 278.84)	221.38±155.19 (91.64; 351.11)	164.13±99.42 (81.01; 247.24)	195.88±52.29 (152.16; 239.59)
Reversible damage				
Thickness (µm)	269.31±107.32 (201.12; 337.49)	429.21±211.21 (252.63; 605.78)	248.22±98.72 (165.68; 330.74)	441.17±107.49 (351.30; 531.03)
Area (µm^2)	801,066.69±370,890.91 (565,413.85; 1,036,719.53)	1,046,609.32±531,069.85 (602,623.82; 1,490,594.82)	575,001.65±316,697.64 (310 235.81, 839,767.50)	1,130,407.54±199.948.63 (963,246.29; 1,297,568.78)
Pores (*n*)	24.25±19.62 (11.78; 36.72)	31.00±23.38 (11.45; 50.55)	22.88±14.00 (11.17; 34.58)	37.13±20.75 (19.78; 54.47)

SD standard deviation, *CI* confidence interval

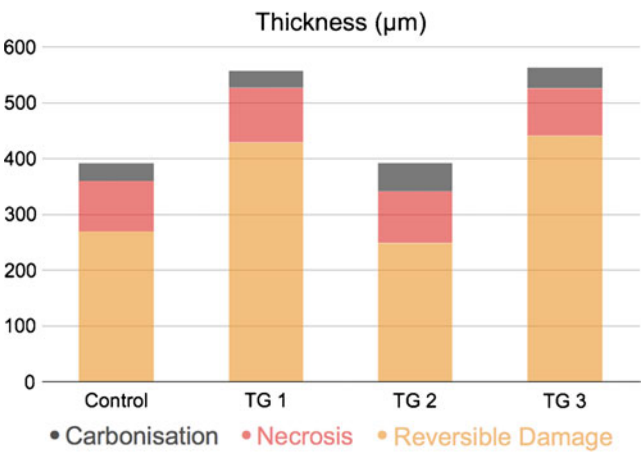

Fig. 3 Mean thickness of damage

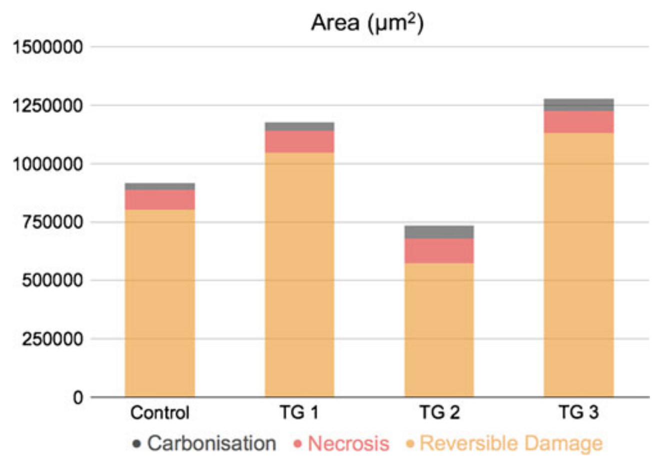

Fig. 4 Mean area of damage

In our study, we used liver tissue, as it fits more to the optical properties of a 980-nm diode laser when compared with bloodless cadaver epithelium while still showing enough similarity to oral tissue; the unpigmented tissue of liver capsule corresponding to the unpigmented lamina epithelialis mucosae. Hemoglobin as the absorbing constituent of the tissue has been shown to be able to uptake oxygen up to 48 h postmortem [31].

The second possibility to minimize collateral tissue damage is to reduce the influence of heat accumulation by applying an external cooling system.

Air cooling for example can reduce the extent of thermal trauma associated with thulium laser surgery of the vocal folds [8].

For CO_2 laser irradiation on human enamel both water and air cooling methods are effective on prevention of thermal damage of pulp [9].

A comparative study about irradiation on porcine oral mucosa using several laser types reported that the samples with the lowest thermal effect were those irradiated with Er, Cr/YSGG laser using water/air spray, followed by CO_2 and diode lasers [10].

The influence of blood circulation as a potential coolant has to be cut out completely in an in vitro study. Still, Niemz et al. reported that heat convection is negligible due to the low perfusion and only play a significant role during long exposure times [32].

Concerning the diode laser, there are no published papers or any kind of diode laser system available taking the benefit of external cooling. The idea of the present pilot study was to combine the increase of cutting efficiency without increase or even reduction of the thermal tissue damage. We investigated the influence of a water/air spray cooling on the collateral thermal damage of diode laser incisions additionally applying an innovative pulse design, which irradiates with intermittent sequence of pulse complexes (micropulsed mode).

Our study clearly showed that incision depth increased significantly under water/air cooling (270.9 versus 502.3 µm—test group 3) without significant changes of incision width.

In test group 2, the total area of damage is significantly smaller than in the control group (in this group the incision depth increased by 65 %).

In test group 3, the incision depth increased by 85 % leaving the amount of irreversible damage almost the same as in the control group. Although the total area of damage is significantly higher, the bigger part of it represents a reversible tissue alteration. The percentage of irreversible damage is significantly reduced in groups 1 and 3 compared with control.

These findings are particularly important for clinical use because reduction of irreversible collateral tissue damage enhances wound healing directly.

As reported, we recognized a quantitative increase in the occurrence of pores throughout the whole damage zones

Table 4 Proportion of thickness/area of irreversible damage to the thickness/area of total damage

Ratio (%)	Control (mean (CI))	TG 1 (mean (CI))	TG 2 (mean (CI))	TG 3 (mean (CI))	*p* value
Thickness irreversible damage/ thickness total damage	33.53 (26.36; 40.69)	25.16 (19.19; 31.13)	38.21 (30.76; 45.65)	22.16 (17.95; 26.26)	0.003
Area irreversible damage/ area total damage	14.91 (10.23; 19.59)	12.80 (7.07; 18.53)	22.83 (16.43; 29.24)	11.80 (8.98; 14.62)	0.009

with significance in the zone of carbonization. As "pores" have never been characterized in literature so far, we tried to explain that as a stir of both, tissue water and cooling water (with its biggest effect on the top tissue zones).

Conclusions

As the diode laser shows to be a good compromise for the daily use in dental offices, finding usage in numerous dental indications (e.g., surgery, periodontics, and endodontics), the minimization of the collateral damage is important to improve the therapeutical outcome.

This first pilot study clearly showed that 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.

Conflict of interest The authors declare that they have no conflict of interests.

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