Bone Regeneration After Peri-implant Care with the CO_2 Laser: A Fluorescence Microscopy Study

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Purpose: *The carbon dioxide (CO_2) laser has been shown to be suitable for the treatment of ailing implants. However, comparatively little is known about bone regeneration after laser treatment. Therefore, the purpose of this study was to determine the course of bone regeneration after peri-implant care with the CO_2 laser.* ***Materials and Methods:*** *In 6 beagle dogs, a total of 60 implants and bony defects were treated either conventionally by air-powder abrasive (group 1), by laser irradiation alone (group 2), or by a combination of the 2 (group 3). After therapy, polychrome sequence labeling was performed using 4 different markers. Four months later, after sacrifice, histologic sections were photographed and scanned. In each specimen, the 4 stained areas were detected with special software and indicated as a percentage of the standardized measurement frame. Lastly, the time-course of the bone regeneration was determined for each of the 3 therapy groups.* ***Results:*** *Fluorescence microscopy demonstrated maximum bone regeneration after 8 weeks in all 3 therapy groups. In this period, groups 2 and 3 showed significantly greater amounts of newly formed bone than group 1 (*$P < .03$ *and* $P < .05$*, respectively). However, there was no difference in bone regeneration between groups 2 and 3.* ***Discussion:*** *Using fluoresence microscopy, it was possible to analyze and interpret the bone regeneration processes during all 4 application phases of the 3 groups.* ***Conclusions:*** *These results support the hypothesis that CO_2 laser irradiation renders significantly more new bone formation, especially 5 to 8 weeks postoperatively, than conventional decontamination in the dog model. Further investigation will be required to determine the clinical efficacy.* Int J Oral Maxillofac Implants 2005;20:203–210

Key words: *CO_2 laser, fluorescence microscopy, implant dentistry, peri-implantitis*

The use of lasers has been shown to be suitable for certain procedures in oral surgery. A new indication might be the sterilization of exposed implant surfaces to rehabilitate ailing implants. However, not all laser systems available in dentistry are of value in this regard.

From the studies of Bida[1] and Block and associates,[2] it was concluded that the use of Nd:YAG lasers in implant-uncovering procedures or peri-implant gingival surgery should be considered inherently unsafe because of laser absorption.[3] In contrast, carbon dioxide (CO_2) laser energy is not absorbed to any significant extent by metallic surfaces, which reduces the potential for damage to the metallic implant surface and for thermal injury to the underlying tissues.[4,5] It has also been shown that CO_2 laser irradiation has good potential for sterilization because of its excellent absorption in water.[6] Therefore, the CO_2 laser has recently been recommended for applications in implant dentistry, including the uncovering of implants at second-stage surgery and decontamination of exposed implant surfaces.[7–9]

Comparatively little is known about the effect of CO_2 laser energy on the regeneration potential of the surrounding bone when this device is used for the decontamination process. A delayed osseous healing

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Table 1 Chronologic Order of Fluorochrome Application

No. of weeks postsurgery	Fluorescent marker	Dose (mg/kg body weight)	Manufacturer
2	Alizarin red complexone	30	Merck, Darmstadt, Germany
5	Terramycin (tetracycline)	15	Pfizer, Karlsruhe, Germany
8	Calcein green	10	Merck
12	Xylenol orange	90	Merck

response with the use of the CO_2 laser has been described in the literature.[10] Even the additional application of cooling water does not attenuate this effect.[11] This seems to be related to the presence of residual char in the osseous defect, which results in areas of heat-induced tissue necrosis.[12] Therefore, the wound healing process after CO_2 laser application lasts longer in comparison to wound healing with conventional treatment methods.

Accordingly, the purpose of this study was to determine the time course for bone regeneration after peri-implant care with the CO_2 laser in a beagle model.

MATERIALS AND METHODS

Dental Laser

A CO_2 laser (model 20 C) manufactured by DEKA (Freising, Germany) was used. The monochromatic light of this CO_2 laser has a wavelength of 10.6 micrometers. Its energy output (5 to 20 watts) can be used in either a continuous, pulsed, or superpulsed mode of laser beam delivery. This means that by changing the frequency of pulses, pulses of many different energy levels, ie, distinct mean power settings, can be achieved.

Additionally, a special handpiece with a focal length of 125 mm was used in this experiment. It produced a sharp spot with a diameter of 200 µm that could be kept in an accurate position during the laser process. A reference pointer mounted to the handpiece allowed precise handling of the laser beam.

The Swiftlase scanner system (Sharplan, Freising, Germany) was also used. This system could reduce the characteristic tissue carbonization caused by CO_2 laser by sweeping a focused laser beam for 0.1 second over an area with an diameter of 3.0 mm (7.06 mm^2). Consequently, the dwell time on each individual point of this area was less than 1 ms.

In Vivo Study

In six 2-year-old female beagle dogs of the same pedigree a total of 60 titanium plasma-sprayed Frialit-2 implants (Friadent, Mannheim, Germany) were placed. The implants were 11 mm long and 3.8 mm in diameter. In each dog, 5 implants were placed on the right side in the premolar and molar regions of the mandible, and 5 implants were placed on the left. After a period of 3 months with oral hygiene maintenance, the implants were uncovered. Cotton floss ligatures were positioned around the implants and left for 3 months to allow gross plaque accumulation around the implants. This resulted in noticeable circumferential peri-implant bone defects which could be assessed in each dog.

Surgical treatment consisted of granulation tissue removal as well as the decontamination of the implant surface with 3 different cleaning methods. Twenty implants (group 1) were decontaminated conventionally by an air-powder abrasive[13] with the Prophy-Jet (Dentsply, York, PA) for 60 seconds. Another 20 implants (group 2) were decontaminated by laser treatment alone (continuous wave, 2.5 W, focus 200 µm, 6 times for 10 s each). Group 3 (the last 20 implants) was treated conventionally using the Prophy-Jet for 60 seconds and then by laser with the same parameters as group 2 for another 60 seconds. Each hemimandible was treated using a single method. Thus, each treatment method was applied in 4 different hemimandibles. After decontamination, the flaps were repositioned and carefully sutured. Over the following 4 months, intravital staining with 4 different fluorochromes was performed (Table 1). This made it feasible to evaluate the time course of bone regeneration.

Histology, Histometry, and Fluorescence Microscopy

The sequential administration of fluorescent markers made it possible to assess the direction and the topographic localization of new bone formation.

Table 2 New Bone Formation 2, 5, 8, and 12 Weeks After Laser Treatment Represented by the Area Stained by the 4 Markers

No. of weeks postsurgery	Average reapposition of regenerated bone (differences between groups)											
	Group 1				Group 2				Group 3			
	Average	SD	Min	Max	Average	SD	Min	Max	Average	SD	Min	Max
2	0.54	1.12	0.00	3.76	0.63	0.69	0.00	3.00	0.68	0.90	0.00	3.54
5	0.38	0.47	0.00	1.55	0.69	1.03	0.00	4.17	0.77	0.80	0.00	3.32
8	2.88	2.59	0.13	8.38	3.82	5.33	0.01	21.60	4.37	4.51	0.25	21.20
12	0.31	0.93	0.00	3.50	1.58	1.62	0.24	6.23	1.70	1.80	0.00	7.22

During a period of 24 hours the fluorescent dyes were incorporated in the mineralization front by means of chelation.[14] Thus the bone markers used in this study could be compared to each other. Following each dye procedure, a characteristic intravital labeling in the new osseous matrix was observable. Therefore, polychromatic fluorescence labeling was performed during bone regeneration after peri-implant care with the CO_2 laser to interpret the bone neoformation according to time. The animals received intravenous injections of the fluorescent markers in a sterile solution as described in Table 1.

After a 4-month healing period, the animal heads were fixed by vascular perfusion with 2% glutaraldehyde following a carotid artery "cut-down" procedure. The mandibles were block-resected, and undecalcified histologic sections were prepared and analyzed according to the technique of Donath and Breuner.[15] The initial section thickness of 300 µm was reduced to approximately 20 µm with the Exakt grinding unit (Exakt Cutting-Grinding System; Exakt Apparatebau, Norderstedt, Germany).

Fluorescence microscopy was performed with a Nikon Mikrophot-FX (Nikon, Tokyo, Japan) device. The accompanying photo documents were produced with a Nikon FX-35 WA camera using Kodak 64 T (Eastman Kodak, Rochester, NY) film. Afterward, 2× magnified colored slides (Ektachrome 100 HC daylight; Kodak) were made of the histologic sections. The resulting transparencies were scanned (Sprint Scan 35; Polaroid, Bedford, MA) using the Micrografics Picture Publisher 4.0 (Microsoft, Redmond, WA) and stored as bitmap data on a personal computer (80486 DX 2/66 16 Mbyte; Intel, Beaverton, OR).

Because the graphics board was equipped with an additional analog-digital converter, it was possible to digitize the analog signal. This was managed by transferring the digital data in a line skip operation, whereby the picture signals were stored in a real-time process on the graphic board. For the whole process, Windows for Workgroups 3.11 (Microsoft) was used as an operating system. With Adobe Photoshop 2.0.1 (Adobe Systems, San Jose, CA), the final bitmap pictures were converted to 8-bit images with 256 colors and analyzed on a 15-inch monitor (Nokia, Bochum, Germany). The digital images were adapted in size to the special analyzing framesize of the graphic software Leica Q 500 MC (Leica, Cambridge, England).

All images were exactly the same size and could be evaluated histomorphometrically in relation to each other. Single stains were defined by their range and density using the software-integrated detection method; thus, the dyes were analyzed in terms of the different qualities and quantities of the colors seen. It was possible to precisely mark the dimensions of the area marked by each label and therefore to assess exactly how much bone regeneration had occurred at every point of time at which a marker had been injected (Table 2). Subsequently, the Leica Q 500 MC graphic software calculated the space marked by the dyes according to the default frame size. The areas marked by the 4 dyes could be measured as a percentage. The results could then be compared, and it was possible to pinpoint the time of highest regeneration for each marker. The data were organized in tabular format.

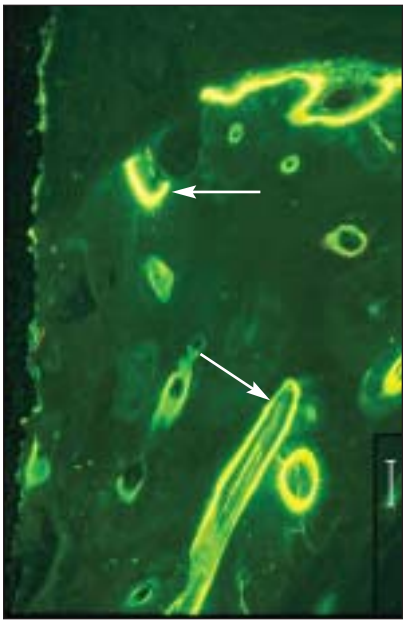
Statistical Analysis

Statistical analysis was performed using Microsoft Excel. A 2-tailed Student *t* test was used to compare the reappositioned bone in the 3 treatment groups. A *P* value less than .05 was considered to indicate statistical significance.

RESULTS

New bone formation was determined histomorphometrically by bone labeling quantification representing the different healing periods after the 3 treatment methods.

Peri-implant bone formation using the air-powder abrasive prophylaxis system was maximized after 8



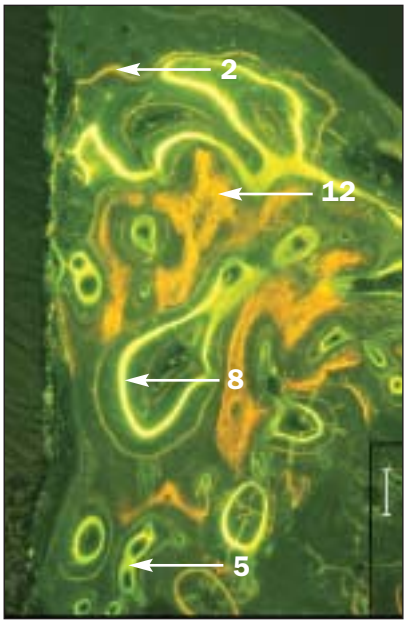
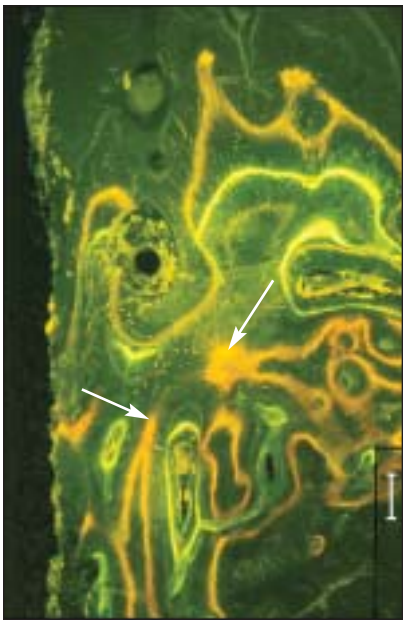


Fig 1 *(Left)* Polychromatic fluorescence labeling after treatment with the air-powder abrasive. The yellow-green stain that is characteristic of bone regeneration after 5 to 8 weeks is prevalent in this example (calcein green; original magnification × 20).
Fig 2 *(Right)* Labeling of peri-implant bone after treatment with the air-powder abrasive. In comparison to Fig 1, all dyes are visible (original magnification × 20).



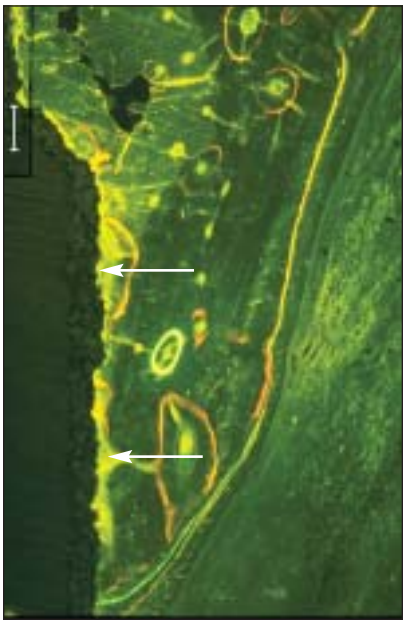


Fig 3 *(Left)* A typical image of stained osseous structures after single laser treatment (group 2) (xylenol orange; original magnification × 20).
Fig 4 *(Right)* Another sample from group 2. Different bands after laser application are less distinctive in comparison to Fig 3. However, the shiny calcein green stain is clearly visible (original magnification × 20).

weeks. Quantitative histomorphometric analysis revealed an enhanced osseous bone formation process during this period. Deposits of the calcein dye could be clearly seen in the vicinity of the implant surface. The shiny green color obtained by calcein staining in a total of 27 measurements showed an amount of apposition of 2.9% (Fig 1). For the other 3 dyes, fluorescence microscopy showed weakly stained bone formation adjacent to the placed implants after 2, 5, and 12 weeks. However, calcein staining was the stain closest to the surface and the label seen most extensively (Fig 2). The "osteoproductive process" after using the Prophy-Jet represented by the amount of detectable dyes accounted for 0.38% in the fifth week and 0.31% in the 12th week. Fluorescent material incorporation occurring after 2 weeks demonstrated with alizarin red complexone staining was 0.54%.

Following CO_2 laser–assisted therapy, the maximum average bone apposition (3.8%) was detected after 8 weeks. The sections with calcein staining showed diffuse yellow and green fluorescence in several places, a pattern characteristic of high bone mineral apposition. They were ubiquitous in the

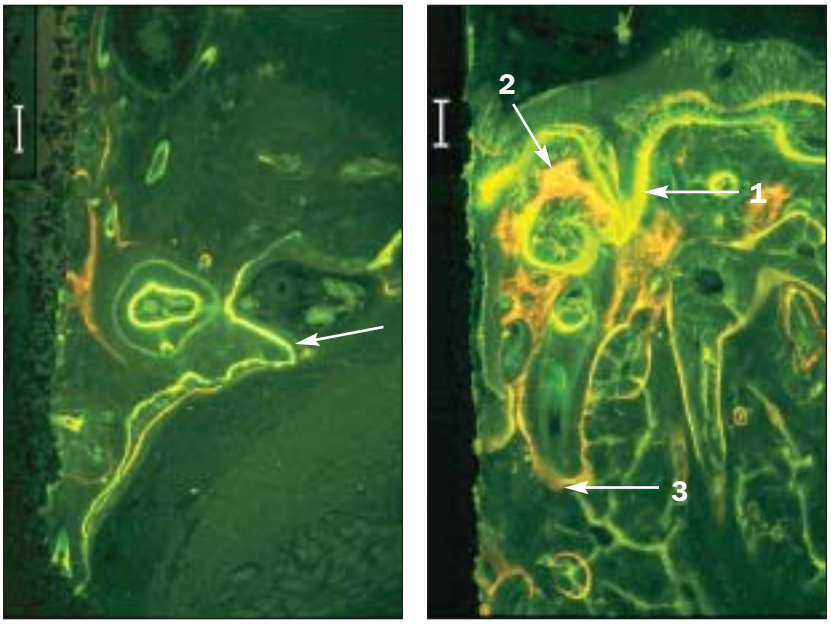


Fig 5 *(Left)* A sample from group 3 after 8 weeks. Although the markers in this image are very weak, the clear and lime-green band of calcein is conspicuous.
Fig 6 *(Right)* This image from group 3 demonstrates the pronounced and specifiable stains after *(1)* 8 and *(2, 3)* 12 weeks.

Table 3 Comparison of the 3 Treatment Groups

Groups	Bone gain		
	Average	Variance	*t* value
1 (n = 27) vs 2 (n = 42)			
After alizarin red complexone application	0.54/0.63	0.87/0.48	.66
After tetracycline application	0.38/0.69	0.18/0.76	.05
After calcein application	2.88/3.82	5.59/20.20	.25
After xylenol orange application	0.31/1.85	0.5/2.09	8.29
1 (n = 27) vs 3 (n = 54)			
After alizarin red complexone application	0.54/0.68	0.87/0.67	.51
After tetracycline application	0.38/0.77	0.18/0.53	.01
After calcein application	2.88/4.37	5.59/15.00	.03
After xylenol orange application	0.31/1.70	0.51/2.64	8.81
2 (n = 42) vs 3 (n = 54)			
After alizarin red complexone application	0.63/0.68	0.48/0.67	.75
After tetracycline application	0.69/0.77	0.76/0.53	.61
After calcein application	3.82/4.37	20.70/15.00	.53
After xylenol orange application	1.58/1.70	2.09/2.64	.70

The average value and variance stand for the amount of detected dye (percentage) of each group.

implant interface; there was dense yellow-green labeling close to the implant surface. At the 2- and 5-week intervals, apposition of bone was 0.63% and 0.69%, respectively. Bone apposition detected with xylenol orange accounted for 1.58% (Fig 3). This marker emitted a strong signal and had close contact to the implant surface. The substances applied earlier (alizarin complexone and tetracycline) were found further away from the implant surface. A clear intensification of labeling could be seen after 8 and 12 weeks (Fig 4).

Combined treatment with the CO_2 laser and the air-powder abrasive system revealed subsequent bone neoformation for 8 weeks. There were slight traces of alizarin complexone (0.68%) and tetracycline (0.77%) after 2 and 5 weeks. Fluorescence microscopic images revealed only weak signals with early phase deposits in the vicinity of the implant surface. After 8 weeks, distinct and extensive labeling of calcein stains (4.3%) with contact with the implant surface could be seen (Fig 5). Staining with xylenol orange (1.7%) was also dense and ubiquitous at the implant-bone interface (Fig 6).

By comparing all therapy groups based on fluorochrome incorporation, a maximum of bone apposition which had its peak from 5 to 8 weeks postoperatively

could be seen (Table 3). During this period results with CO_2 laser (0.69%) and the combination of both treatment modalities (0.77%) were significantly better than therapy with air-powder abrasive alone (0.38%). There was significant statistical difference when comparing the presence and quantity of fluorochromes at this application period. The measurable fluorescent material incorporation was much more dense and extended when the laser was applied than in the case of air-powder abrasive application. Thus the process of osseous transformation at the bone-implant interface was significantly improved. However, direct comparison between groups 2 and 3 did not show substantial differences between the 2 groups at week 5 ($t = .61$) or week 8 ($t = .53$). Fluorescence labeling did not reveal a statistically significant distinction between them at this application period. Fluorochrome incorporation slightly increased until the 8th week. However, when the 2 groups were compared, there was no significant difference in the amount of dye incorporation in the bone. In groups 2 and 3 the deposit of fluorescent material decreased again up to the 12th week.

When focusing on the other labeling phases in comparison to the air-power abrasive, only a slight, insignificant enhancement of bone tissue formation around the dental implants was observable. Histomorphometrically, the bone marker quantification revealed no significant statistical difference. Consequently, the only significant advantages of CO_2 laser application for peri-implant care in this model were limited to a time period of 5 to 8 weeks postoperatively.

DISCUSSION

Under the conditions of this study, CO_2 laser–assisted decontamination of exposed implant surfaces had no adverse effects on the reosseointegration of so-called ailing implants in the dog model. The results of this vivo study, however, demonstrate that the CO_2 laser parameters chosen for decontamination can be considered "safe" for such procedures and for the regenerative capacity of the surrounding bone. The results of the fluorescence analysis showed that bone tissue was formed during all 4 application phases in all 3 groups. Even though the differences in the osseous processes were quite small during the initial administration period, a tendency toward slightly better reosseointegration after single or combined CO_2 laser application could be seen. This was confirmed by the detected apposition of stained bone. However, a significant distinction could not be proven. Since no further fluorescence microscopy data about bone regeneration after peri-implant care with the CO_2 laser could be gleaned from current literature, the results of the present study should be verified by similar investigations. Currently histomorphometric and fluorescence microscopic analysis of bone remodeling adjacent to dental implants mainly focuses on static load[16] and implant surface[17] and shape.[18] Yet these data can also provide vital information for understanding the course of osseous processes in the vicinity of formerly osseointegrated implants, because fluorescence labeling phenomena of the bone after ligature-induced peri-implantitis treated with the CO_2 laser are not distinctive from usual osseous neoformations. In all cases, quantitative histomorphometric analysis made reosseointegration visible by characteristic stains more quickly. For this reason it was possible to analyze and interpret the results of the 3 therapy groups.

In the present investigation, a significantly greater amount of woven calcified matrix was seen 5 to 8 weeks postoperatively in groups 2 and 3 than in group 1. The typical green stains showed a different pattern, and deposits of the fluorescent dyes were much more dense. During this period bone regeneration reached its peak; the methods used in groups 2 and 3 appeared to be much more effective than conventional treatment. By comparing these data with other treatment methods for induced peri-implantitis[19,20] it becomes obvious that the use of CO_2 laser can be successful for the peri-implant care of ailing implants. The laser not only removes granulation tissue but also vaporizes[21] any bacteria. Hence, laser therapy can be superior to decontamination methods that generally achieve a minimum reduction of the bone defect.[22]

Additionally, this histomorphometric study showed the ability of the laser beam to induce tissue regeneration. However, the conspicuous osseous effects of the CO_2 laser could not be determined for the whole period of histomorphometric observation. After the maximal bone regeneration, represented by the light green calcein stain, had been reached after 8 weeks, the osteogenic process slowed down in every group. Moreover, no statistically significant differences could be found 12 weeks postoperatively between groups 1, 2, and 3. The positive sterilization effect of the CO_2 laser had already lost its influence, which led to a decreased healing process. In this case laser treatment would be similar to conventional decontamination devices[23] such as scalers or citiric acid, which are also commonly employed in periodontology.[24,25] Further investigations will be required to understand the significant gain of clinical attachment after 5 to 8 weeks with the single CO_2 laser application and the rapid slow-down after this period. Also, the differences in the combination treat-

ment method and the single CO_2 laser use during this period need more detailed study.

As it is commonly known from hard tissue laser ablation, the earliest provable bone formation after laser treatment can be analyzed in the second and third week postoperatively. Then bone change procedures have progressed almost within the range of the entire bone. This has been demonstrated in the literature.[26] Other studies have shown that the thermal effects of the laser can cause pronounced tissue necroses.[27] The laser ablation of bone tissue generally leads to the formation of a carbonization layer, which obviously delays wound healing. Although the CO_2 laser parameters were not coordinated with hard tissue ablation settings in this study,[28] the biologic effects that developed during the irradiation of bone with the CO_2 laser could be comparable to the present wound healing results. This could explain the peak of osseous neoformation after 5 to 8 weeks in the present study.

SUMMARY

It may be summarized that the CO_2 laser treatment can be an acceptable alternative to conventional cleaning methods, since greater bone regeneration can be achieved without the use of additional treatment methods such as the submerged membrane technique[29] or guided tissue regeneration.[30] However, the positive effects of the CO_2 laser could only be demonstrated for a limited period of time. Further comparative studies are necessary to analyze whether other laser parameters or even other lasers, such as the erbium laser, can lead to better results in peri-implant care.

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