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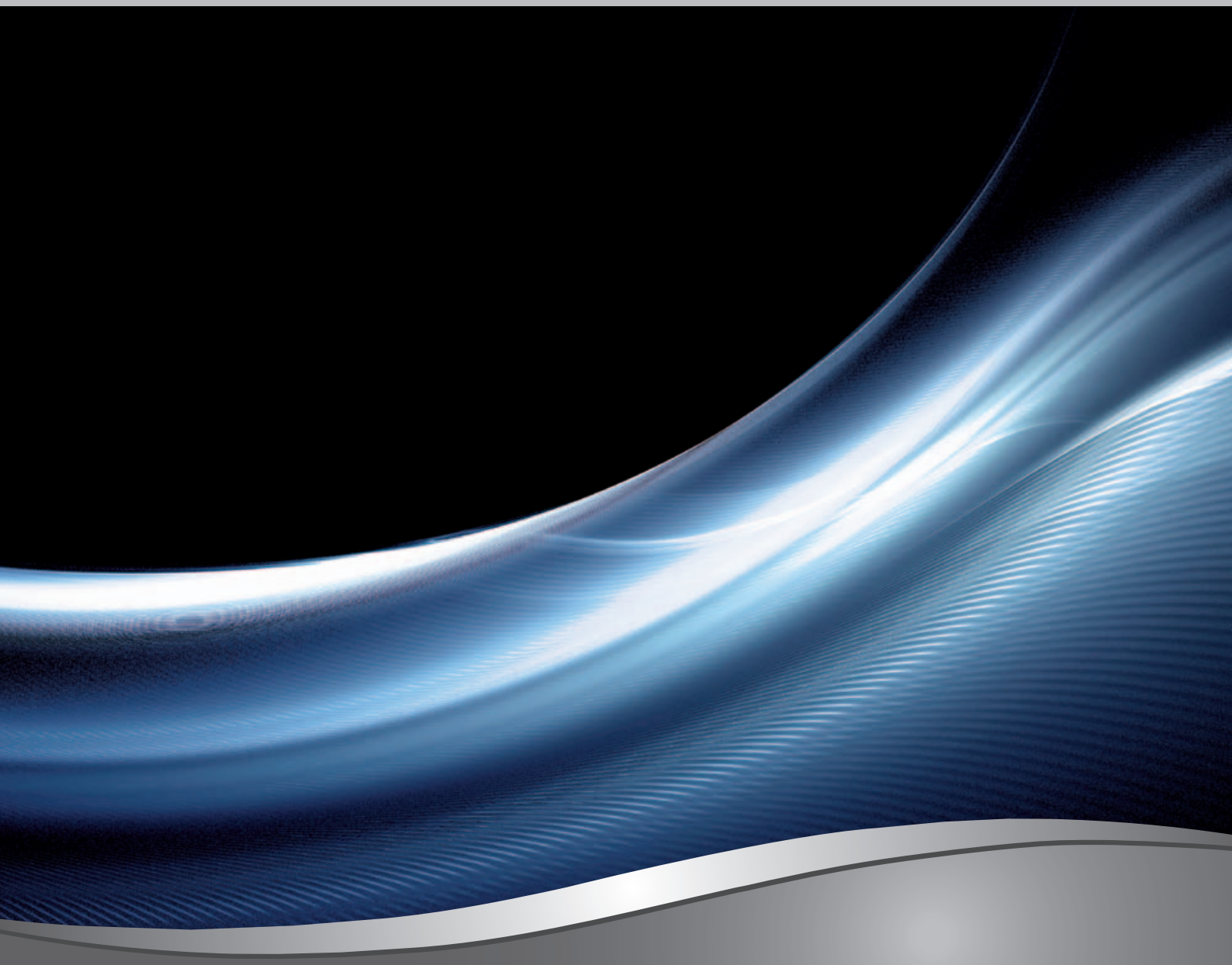

selected implantology studies

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A Novel technique of laser-assisted blood coagulation for tissue regeneration in implant dentistry

author_Kenneth Luk, Hong Kong

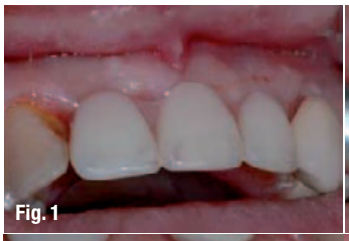


Fig. 1

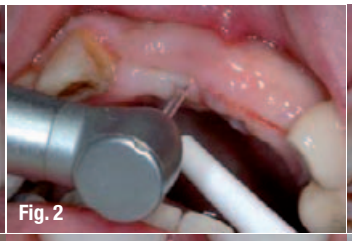
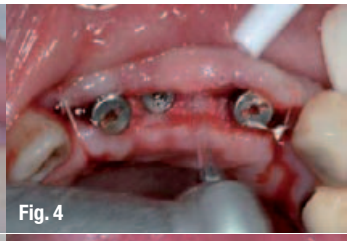


Fig. 2


Fig. 3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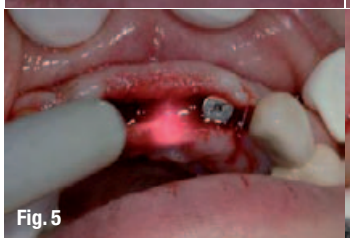
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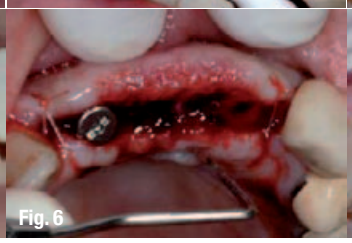
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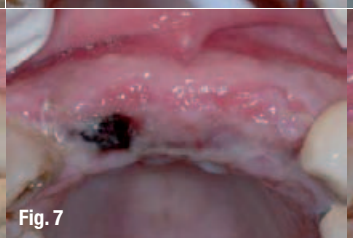
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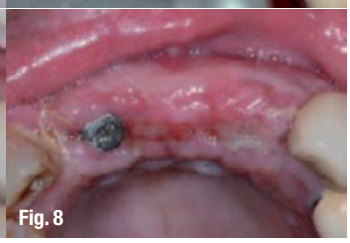
Fig. 8

_Abstract

Various laser wavelengths have been demonstrated in assisting implant surgery such as uncovery of implant sites, flap incision, gingival management in restorative phase. Recently, researches in treatment of peri-implantitis and preparation of osteotomy sites with Erbium-doped:Yittrium-Aluminium-Garnet (Er:YAG) lasers have been reported. The Er:YAG laser is used for ablation of dental hard tissue and bone with the benefit in decontamination and removal of smear layer. Er:YAG laser also ablates soft tissue efficiently with low collateral thermal damage but poor in haemostasis. Haemostasis, coagulation and biostimulation in soft tissue management are major advantages in the use of diode laser with 810 nm wavelength. The aim of this case report is to demonstrate the effect of laser-assisted blood coagulation (LBC) on soft tissue regeneration in a space between opened flaps prepared by intentional flap-positioning around implant.

A combination of two lasers, digital pulse diode laser (DPL) 810 nm and Er:YAG laser 2,940 nm were employed for the LBC technique. Fibroblastic proliferation covering the entire wound was observed two days after treamtment. The increase in tissue bulk at the pontic areas improved the emergence profile and aesthetics of the bridge and soft tissue support. In this case, palatal connective tissue graft was avoided. The LBC technique is a useful adjunct to tissue/wound management and holds a promise for tissue regeneration.

_Case Outline

A 40-year-old lady attended the office for restorative phase of implant (Fig.1). Three implants were placed on 11, 21 and 22 by her maxillofacial surgeon (Dr. Richie Yeung) five months ago. A removable acrylic denture was worn by the patient during the healing phase.

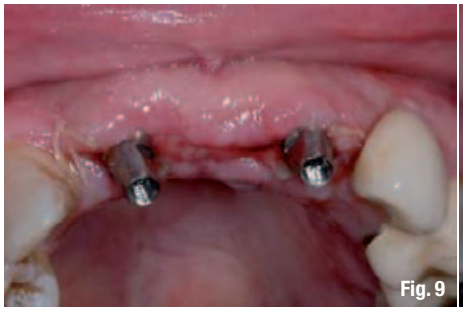
Fig. 9

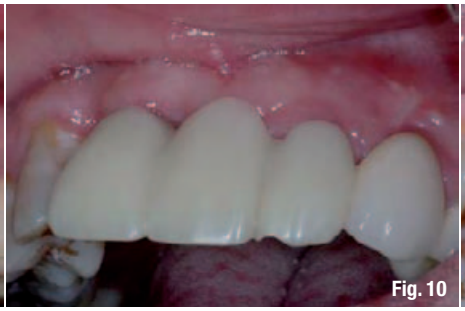
Fig. 10

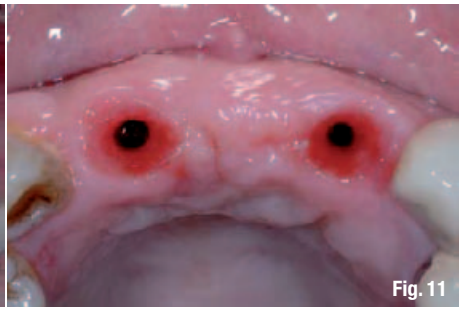
Fig. 11

_Treatment Plan

I. Incision with Er:YAG laser
II. Exposure of implants and placement of gingival former
III. Induce bleeding into open wound
IV. Haemostasis and coagulation with DPL.

_Treatment procedures

Elexxion Dental Laser (Delos) was used in this case report. This is a combination laser unit housing both Er:YAG 2,940 nm laser and DPL laser. Local anaesthetic was administered. Incision was made on the ridge of 11 to 22 sites by Er:YAG laser (2,940 nm) under water irrigation at 70 mJ/100 µsec pulse and 20 Hz using a 400 µm tapered sapphire tip (Fig. 2). Full thickness flap was raised with periosteal elevator. The flap was loosened along the buccal and palatal side of the ridge (Fig. 3). It was decided that only two implants were to be used as abutments. Gingival formers (3 mm in height by 4 mm diameter) were placed at 11 and 22 sites.

One suture was placed at each end of the flap. The flap was intentionally kept open supported by two gingival formers without sutures in between. Gingival mucosa of the flap was de-epithelialized by Er:YAG laser with water irrigation at 70 mJ/pulse and 20 Hz using a 400 µm tapered sapphire tip (Fig. 4). The periosteum was also ablated by Er:YAG laser to induce bleeding to fill up the open wound. Relieving incisions were also made with No.15 scalpel to induce sufficient blood volume. Blood was coagulated by DPL at 20 W, 16 µsec and 20,000 Hz in de-focused mode using a 600 µm fiber (non-initiated) (Fig. 5). Coagulation (pink in colour) may be observed while avoiding charring (black in colour) on the surface of the clot (Fig. 6).

_Post-operative Care

The patient was asked not to disturb the clot while wearing the removable denture at all times. Toothbrushing near the site should to be avoided.

Warm salt mouth bath was recommended. Patient was advised not to use antiseptic mouth rinse. No antibiotics or analgesics were prescribed.

_Result

Patient reported no adverse signs or symptoms. Fibrin mash covering the entire wound was observed two days after treatment (Fig. 7). The gingival former for 22 was covered by the newly laid fibrin while the remains of the clot was still covering the gingival former at 11. On day three, impression was taken for the fabrication of provisional bridgework (Fig. 8 & 9). Five weeks post-op showed the profile of the provisional bridgework (Fig 10). Three months (Fig. 11) and six months (Fig. 12) post-operative reviews showed complete keratinisation of the soft tissue (Fig 13). The patient was happy with the aesthetics of the screw-retained prostheses (Fig. 14).

_Conclusion

The increase in tissue bulk at the pontic areas improved the emergence profile and aesthetics of the bridge and soft tissue support. In this case, palatal connective tissue graft was avoided. The LBC technique was very effective for tissue regeneration with minimal side effects and complication. The LBC technique is a useful adjunct to tissue/wound management and holds a promise for tissue regeneration._

_contact laser

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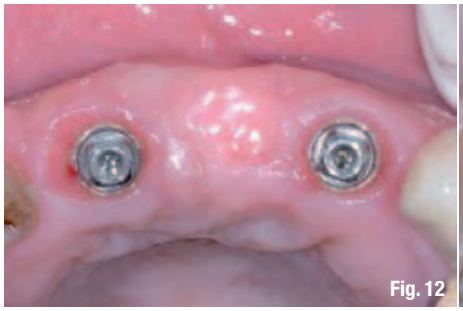


Fig. 12

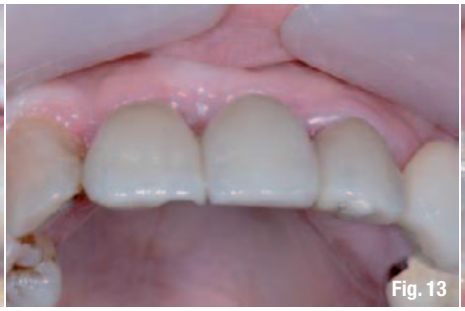


Fig. 13

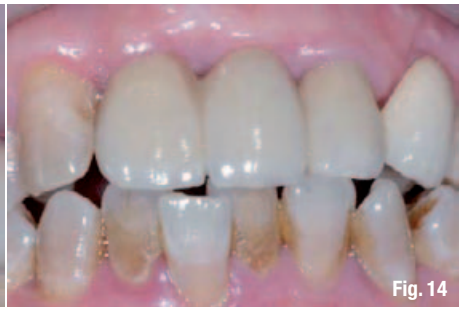


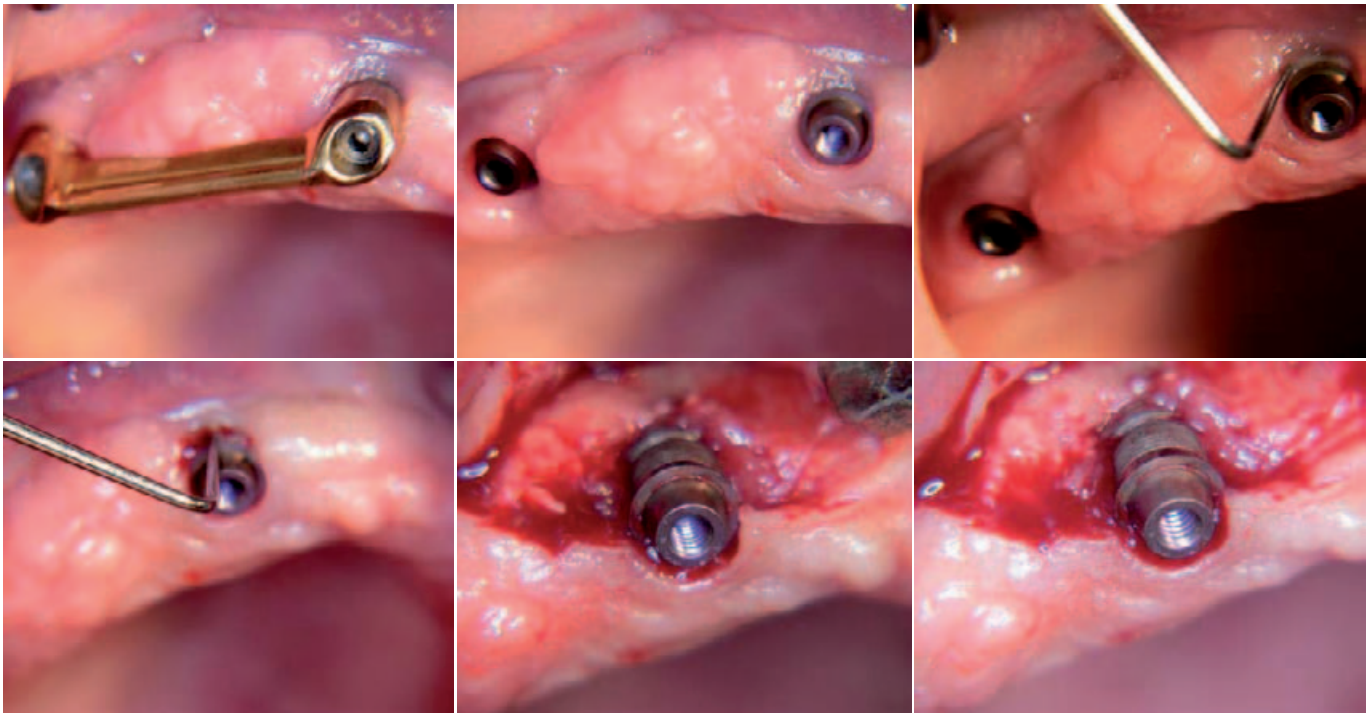
Fig. 14

Integration of diode laser surface decontamination in periimplantitis therapy—a twelve year review of a fit for practice concept

author_Georg Bach, Germany

Manifestation of periimplantitis
On probing, secretion is released at the mesial implant, though the clinical appearance is inconspicuous and further probing leads to a substantial bleeding. After mobilization of the soft tissues, the typical crater-shaped periimplant bone defect becomes visible.

_After many years of great euphoria, a certain disillusion has spread in implantology, which is especially due to the reason that implants with corresponding suprastructures do not last forever, like it has often been pointed out. Anyway, complications cannot totally be excluded. Professor Herbert Deppe, Chair for the Dental Surgery and Implantology Department of Munich University, has recently reported on the fact that approximately an eights of incorporated implants show periimplantary lesions after about 10 years. In the beginning, the main fear was that enossal implants had to face early complications. Nowadays, this is no more the case since sophisticated surgery techniques and improved implant surfaces have reduced these risks. One still has to worry about long-term sequelae shown in artificial abutments caused by periimplantary lesions after some years of strain. However, periimplantitis is mainly induced by bad oral hygiene and/or the inability to carry out mouth care (eg in old patients), and it is not associated to a certain type of implant (system-independent). Numerous therapy approaches have been made to preserve artificial abutments suffering from periimplantitis. A four phase treatment model is usually applied (hygienization phase, surgical resective phase, reconstructive and augmentative phase, recall phase). This model has considerably been enhanced by the launch of diode or injection lasers, which have



later been complemented by CO_2 laser, Er:Yag laser and Er,Cr:YSGG laser respectively. Since the mid-nineties, diode lasers belong to the established wavelengths used in dentistry. Today, diode lasers with short pulse technique are predominant, though it all started out with the cw mode. High performance diode lasers emit monochromatic, coherent light of wavelength 810 nm, which is especially well absorbed by dark surfaces. Thanks to these physical conditions, the injection laser (= diode laser) is perfectly suitable for incisions applied in standard dental surgery, as well as for the resection of benign tumors in the oral cavity, the uncovering of implants and for application in mucogingival surgery. The good cutting properties of diode lasers are due to the extraordinary absorption of laser light by the hemoglobin located inside the tissue. Additional to soft tissue surgery, the diode laser is also used for decontamination of surfaces coverd with microbes (on implants and teeth). It could be demonstrated that especially the gram-negative, anaerobe microbiological spectrum was properly damaged by laser light (Bach und Krekeler (1995; 2000)). In compliance with reasonable peformance and time parameters, which have been confirmed sustainably by clinical long term studies (Moritz (1996), Gutknecht (1997), Bach et. al. (1995, 1996, 1998, 2000, 2001)), a thermic or morphological damage of the implant surface and the surrounding bone tissue can definitively be excluded (Bach and Schmelzeisen (2002)). It was the aim of the present study to demonstrate and evaluate a treatment model for periimplantitis therapy, which shows sustainable results and which is absolutely suitable for practice. There is no doubt that the conventional methods for periimplantitis treatment, which have often been described in literature, permit adequate surface cleaning and thus also the reduction of pathogenic microorganisms on the implant surfaces. Nevertheless, the complete removal of relevant bacteria cannot be ensured. Moreover, the conventional removal of biofilms has only little influence on those bacteria infiltrating the soft tissue. The integration of diode laser light in periimplantitis therapy must be seen as a new approach.

_Material and method

Ten patients (with n = 17 implants) have been treated and examined for a period of more than 12 years (since May 2007). In spring 1995, all of them suffered from periimplantitis on their artifical titanium abutments.

_Pathogenesis of periimplantitis

Periimplantitis therapy represents a border area between implantology and parodontology. The causes for parodontitis and periimplantitis are bacterial infections, in particular they are biofilm based infectious diseases. Gram-negative and anaerobe microbes are mainly responsible for the destruction of the parodontal and periimplantary supporting tissue. As a rule, one of the following microbes causes parodontopathy in case of one of both biofilm based infectious diseases:

_ *Actinobacillus actinomycetemcomitans*
_ *Prevotella intermedia* and
_ *Porphyromonas gingivalis*

Whereas periimplantitis is mainly caused by the following microbes:

_ *Fusobacteria*
_ *Prevotella intermedia* and
_ *Porphyromonas gingivalis*

The principal object of periimplantitis therapy carried out in our dental clinic was to remove the biofilm and hence the removal of the mentioned pathogenic microorganisms.

_ Patients treated

For detailed data, age and sex of the patients, please see Figs. 1 and 2. It should be mentioned that an accumulation of the diseases first incidence is registered in the middle years (age: 30 to 50 years) in both groups. Sex-specific differences could not be ascertained.

Age	Number of patients
20–30 years	1
30–40 years	3
40–50 years	3
50–60 years	2
60–70 years	1

Fig. 1_ Age pattern of the examined and treated patients in 1995.

Sex	Number of patients
Female	5
Male	5

Fig. 2_ Evaluation according to the sex of the examined and treated patients.

_Inclusion and exclusion criteria

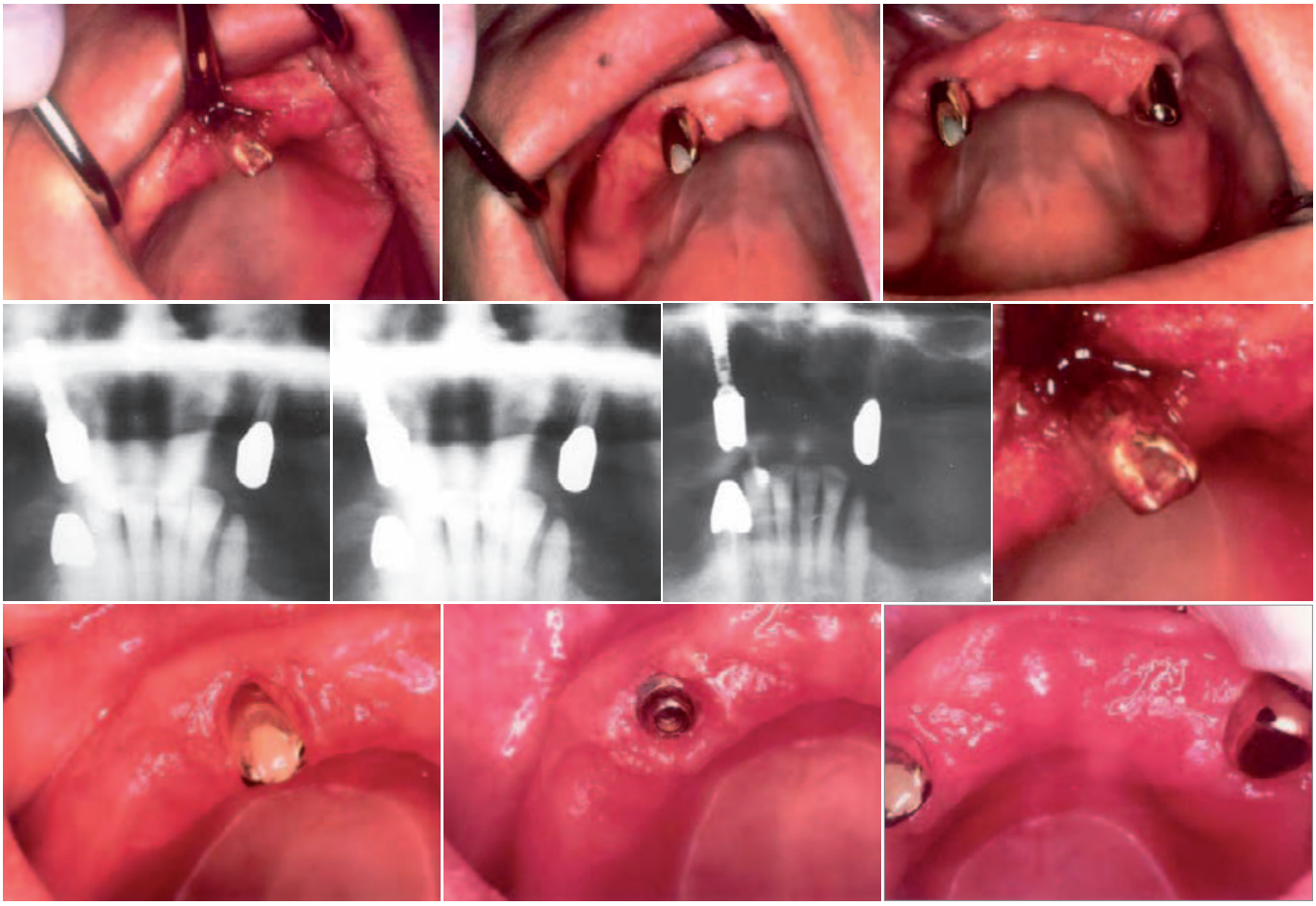
All patients involved had to meet strict inclusion criteria as there were:

_ Clinically visible inflammatory signs like BOP (bleeding on probing) and high probing depths
_ Radiovisible periimplantary bone lesions ("crater")

Exclusion criteria were:

a) Severe primary diseases
b) Nicotine or alcohol abuse
c) Lack of compliance

Due to the strict inclusion and exclusion criteria only a limited number of people could be admitted for this study.



Periimplantitis therapy: You can see the first patient, who had undergone periimplantitis treatment, by means of diode laser decontamination, according to our model. November 1994: Manifestation of periimplantitis at implant regio 13. The panoramic tomography (detail) shows a significant bone loss at the artificial abutment. After mobilization of the soft tissue the situation of the defect becomes clearly visible. January 2008: The prothesis made in 1990, is still in the same positition. The situation of the treated regio 13 implant does not show any irritations with and without suprastructure. There is no evidence of probing depth. The panoramic tomography shows a stable bone situation. Besides the reconstructed defect of regio 13, only the root filling of 43 protrudes. This is the only difference compared to the tomography taken in 1995.

_Treatment procedure

Equal treatment procedures for all periimplantitis patients:

1. Initial therapy:

_ Motivation and instruction of patients
_ Cleaning and polishing
_ Application of desinfecting agents

2. Resective phase:

_ Forming of a mucoperiostal flap
_ Removal of granulation tissue
_ Decontamination by means of diode laser light ($p = 1.0$ watt, $t_{max} = 20$ sec.)
_ Apical shifting of soft tissues

3. Reconstructive phase:

_ If necessary, bone augmentation
_ Where applicable, mucogingival corrections

4. Recall phase:

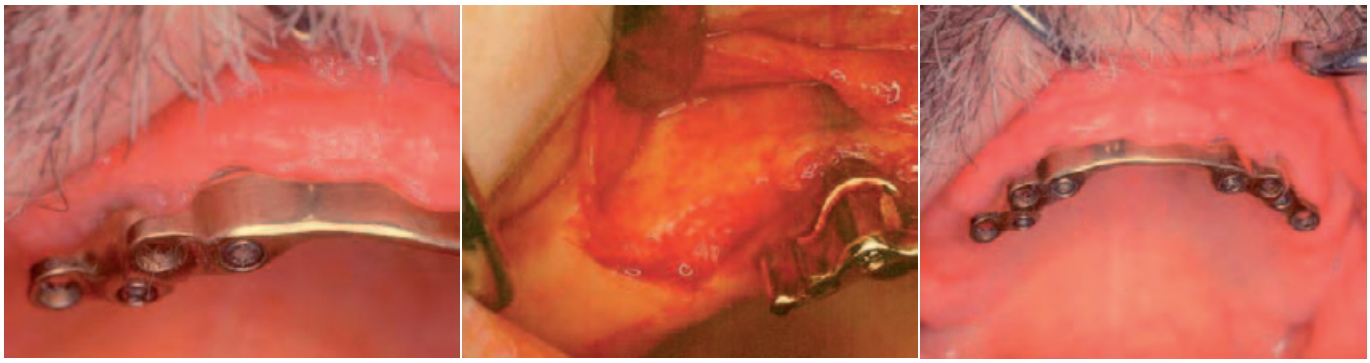
_ After four weeks, six months, one year and then annual evaluations of clinical findings, taking of X-rays (PSA), decontamination of eventually exposed areas by means of diode laser light.

_Image processing methods

As a rule orthopantomograms (panoramic tomography) and additionally dental films in parallel technique were chosen as an adequate image processing method. In some cases of exacerbated inflammations A/B scan ultrasonic methods were applied. A preoperative orthopantomogram and the dental film status (dental shots of the respective areas) were taken. A postoperative orthopantogram was directly taken after surgery. A panoramic tomography was taken one year later and then every two years. The advantage of the orthopantomogram is its panoramic-like view of all teeth, the osseous limbus alveolaris and important neighbouring anatomical structures. The dental film in parallel technique allows statements concerning progredience, stagnation of loss of hard and soft tissue, and it shows the course of the limbus alveolaris in a reproducible way.

_Microbiological diagnosis

Time schedule: Preoperative, four weeks postoperative, one year postoperative and in a 5 to 10-year postoperative interval germs were eliminated from the effected areas. We did not apply the classical microbiological examination technique (isolation of microbes—cultivation—pure cultures—microscopic samples—gas chromatography—antibiotic sensitivity testing—and biochemical identifi-cation, the so-called "bunte Reihen/colour ranks"). We used DNA-RNA hybridization probes instead. The advan-



tage of these hybridization probes is that no living material of the areas probed is needed for cultivation purposes, which minimized the work in the dental clinic (without direct access to an Institute of Microbiology). Additionally, the results were much faster on hand as is the case with classical microbiological examinations. The disadvantage of this rapid test is its high price. Furthermore, only special marker microbes can be detected and **not all** pocket microorganisms can be determined. The germ extraction site had to be dried carefully with a cotton swab, the paper tip was placed, and after a waiting time of 10 seconds put into a sterile storage vessel and sent to the manufactoring company for microbiological diagnosis. The company is in charge of microbiological diagnosis and evaluation of the so-called microbe marker values. The classification of marked microbes was: less than 0.1 % = negative; 0.1-0.99 % = low; 1.0-9.9 % = middle, more than 10 % = high.

_Laser light decontamination

Decontamination formed an essential part of the whole therapy. It was carried out by means of diode laser light with 1 watt performance and 20 seconds of application time per implant under fiber contact. A special program (I = implantology-parodontology) was at our disposal, which was used together with the corresponding device (Oralia 01 IST). Performance and time limitation (1.0 watt, 20 seconds) were already fixed parameters of this program. When observing these parameters (time limitation and limitation of performance) it can be guaranteed that the disease causing microbes will be damaged sufficiently and thus, pulpa, periimplantary and periodontal tissue structures will not suffer any thermic damages (Bach and Krekeler (1995)).

_Results

Alltogether 10 patients could be examined and checked up during the whole 12 years. In 1994/1995 the "Diode Laser Basic Study" of the Department of Periodontal Surgery of the Dental Clinic in Freiburg/Germany included 50 periimplantitis patients. Due to moving, change of dentist, dead of patients and other unknown reasons the number of patients was reduced to 10, who are still patients of my dental clinic.

a) Microbiological results

For microbiological results please see Fig. 3. It must especially be emphasized that *Porphyromonas gingivalis* could nearly be completely eliminated during the whole examination period, and a significant reduction of other anerobe, Gram-negative bacteria could be achieved. We could obtain similar results for *Porphyromonas gingivalis* and *Fusobacteria* except for two cases of low concentration and one of middle concentration, these bacteria could be limited to the lower level of detection in other patients, whereas other relevant marker microbes could be considerably repressed.

b) Recurrence

One of the following results was considered to be a case of recurrence:

_ Occurrence of probing depths of more than 4 mm
_ Loss of implant
_ Recurrence of an inflammation
_ Excessive soft tissue inflammation with pocket activity

After 12 years the quota of recurrence was 23% in the periimplantitis group (4 implants). It is stated in international literature that the five year observation period recurrence rate is 30 %.

c) Losses after 140 months

Within the examination period of 12 years we suffered the following losses: two of 17 implants (12 %).

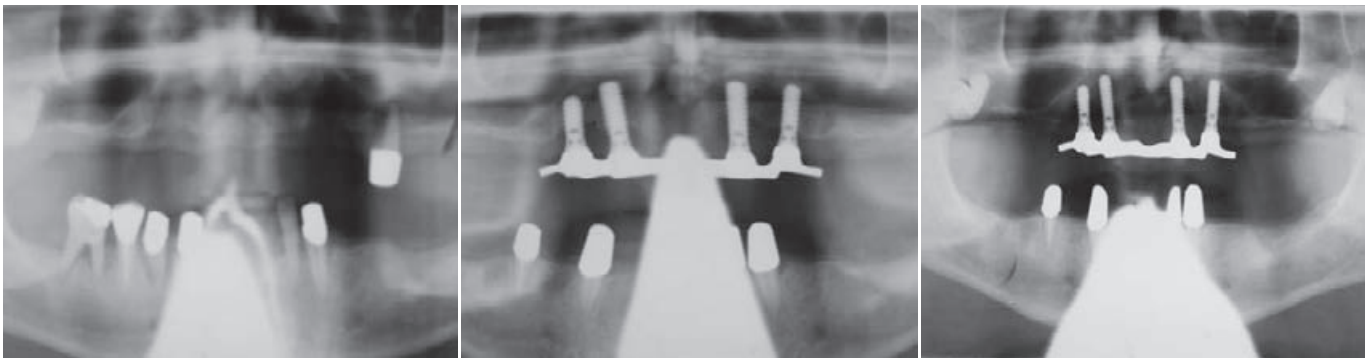
d) Radiological results

On the occasion of the one year check up, a reconstruction of the once crater-shaped defect could be found at the first thread and implant cervix respectively in all 17 implants. After five years this was the case in twelve implants, after ten years in ten implants and in nine implants, when the last X-ray control was carried out. In two implants a successive loss of the bony supporting tissue forced us to remove the artificial abutment in one case af-

Saving of a prothesis by treating periimplantitis of a strategically important implant in the upper jaw.

March 1995: Just one year after the incorporation of a very sophisticated and for the patient nearly too expensive implant-supported prosthesis in the upper jaw, the manifestation of periimplantitis was detected in the first quadrant. After mobilization of the soft tissue (below) the defect situation becomes clearly visible. Four months after the surgical resective phase there were no clinical signs of irritation.

November 2007: The protesis is still in its intraoral place. Meanwhile, the patient has reached the age of 63 years. The situation of the treated implant regio 13 (total suprastructure) does not show any clinical signs of irritation in toto and in the former surgical area. There is no probing depth.



The radiological situation
We are lucky to have a panoramic tomography, taken by the pretreating dentist/referral dentist, which shows the situation BEFORE the implant´s incorporation. Please note the profound parodontal lesions (above). March 1995 (below): Only half a year after incorporation, a considerable bone loss at the artificial abutment (below) can be seen on the panoramic tomography (detail). Another half a year later (upper right) it has drastically expanded and also affects the mesial implant. This was the date, when the patient was referred to our dental clinic. The bony situation seems to be stable when looking at the panoramic tomography from 2006. Besides the 2/3 reconstructed former defect of regio 14, the nearly completely stable reconstruction of the implant regio 13 is certainly impressive.

Fig. 3_ Development of PI microbe marker values 1995–2005.

ter seven and in another case after nine years. After the last X-ray control, the remaining six implants showed losses of horizontal supporting tissue on the level of the first/second thread.

e) Importance of the present study for patients

Two implants got lost and together with their excorporation the corresponding continuous beam (in one case) and bridge reconstruction (in the other case) had to be removed as well. All the other treated implants are properly functioning, even 12 years after periimplantitis detection. Though, at the moment, not all implants are in stable conditions and again bony losses occurred after years of therapy, as already described in the Result chapter, it can certainly be judged favorably that most implants could be preserved. This is of special importance to older patients, who were not in favor of explantation, augmentation and renewed implantation or whose state of health would not have allowed or at least would have limited these highly invasive measures.

_Discussion

The authors considered the 12 year lasting continuation of this study as necessary in order to show that periimplantitis therapy can also be carried out successfully "on the conditions of a private dental clinic". It also proves that "sooner or later not every periimplantitis" leads to the loss of the artificial abutment. The extremely long term of the study was a limiting factor for the contingent of patients involved in examination and treatment. The low number of patients participating in this study was due to the strict inclusion and exclusion criteria. Nevertheless, these severe restrictions helped to minimize the risk of probable influences on the results by external factors. After years of incessant euphoria in implantology, nowadays dentists have to face a considerable number of complications. In my opinion, periimplantitis is the main challenge for today's implantology. The huge number of incorporated abutments and the growing age of our patients, which is one of the main reasons for the increasing loss of ability to handle and clean complicated suprastructures, will lead to a progredience in periimplantitis. The chief purpose of the systematic therapy in case of apparent periimplantitis is the removal of biofilm and pathogenic microorganisms (biofilm management). On the basis of the present results of implant surface decontamination, we believe the integration of diode laser decontamination to be a tried and tested means for treating periimplantitis. Additionally, it implicates a considerable lowering of recurrences and a notable improvement for the prognosis of this clinical picture._

The reference list can be requested from the editorial office.

_contact laser

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Date:	preoperative	4 weeks p.o.	1 year p.o.	5 years p.o.	10 years p.o.
Microbes					
Fusobacteria	2n/3m/1h	2n	2n	2n/1m	2n/1m
Prevotella intermedia	4n/2m	1n/1m	2n/1m	2n/2m/1h	2n/2m
Porphyromonas ging.	2n/4m/2h	1n/1m	2n/1m	2n/2m	2n/2m

(Legend: k.N. = no findings; n = low; m = middle; h = high)

Lasers Med Sci
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ORIGINAL ARTICLE

Decontamination efficacy of erbium:yttrium–aluminium–garnet and diode laser light on oral *Candida albicans* isolates of a 5-day in vitro biofilm model

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Abstract The different forms of superficial and systemic candidiasis are often associated with biofilm formation on surfaces of host tissues or medical devices. The biofilm formation of *Candida* spp., in general, necessitates significantly increased amounts of antifungal agents for therapy. Often the therapeutic effect is doubtful. A 5-day biofilm model with oral *Candida* isolates was established according to Chandra et al. [39](J Dent Res 80:903–908, 2001) on glass and titanium surfaces and was modified by Sennhenn-Kirchner et al. [40](Z Zahnärztl Implantol 3:45–51, 2007) to investigate different aspects unanswered in the field of dentistry. In this model, the efficacy of erbium:yttrium–aluminium–garnet (Er:YAG) light (2940 nm, 100 mJ, 10 Hz, 300 μs pulsed mode applied for 80 s) and diode laser light (810 nm, 1 W, continuous wave mode applied for 20 s with four repetitions after 30 s pauses each) was evaluated and compared to untreated controls. The photometric evaluation of the samples was completed by observations on morphological changes of yeast cells grown in the biofilm. Compared to the untreated controls *Candida* cells grown in mature in vitro biofilms were significantly reduced by both wavelengths investigated. Comparison between the different methods of laser treatment additionally revealed a significantly greater effect of the Er:YAG over the diode laser. Scanning electron microscopy findings proved that the diode laser light was effective in direct contact mode. In contrast, in the areas without direct contact, the fungal cells were left almost unchanged. The Er:YAG laser damaged the fungal cells to a great extent wherever it was applied.

Keywords Oral biofilm · In vitro model · Laser light · Surface decontamination · *Candida albicans* biofilm

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Introduction

Manifestations of candidiasis are associated with biofilm formation occurring on surfaces of host tissues and medical devices [1, 2]. *Candida albicans* is the most frequently isolated causative pathogen of candidiasis [3], and the network of the biofilm displays significantly increased levels of resistance to conventional antifungal agents [4].

The dimorphic yeast *C. albicans* can be either a commensal or an opportunistic pathogen that can cause a variety of infections, ranging from superficial mycoses to life-threatening illnesses [5].

In elderly patients, oral *Candida albicans* strains occur at a frequency above average, especially in patients wearing dentures [6, 7]. These dentures are frequently combined with dental implants. A causal relationship between a persisting biofilm on the implant surface and the occurrence of peri-implant inflammation has been clinically established. The proof of colonisation of certain bacteria and yeasts was associated with peri-implant infections, in some cases even with loss of implants [8–12].

One concept for prevention and therapy of peri-implant infections is the decontamination of the surface, which leads to a reduction in the number of pathogenic microbes on the implant surface [12]. In cases of biofilm-associated infections with fungi it is important to increase the efficacy of treatment. The reason is a reduced susceptibility of *C. albicans* towards conventional treatment approaches [4].

The antimicrobial activity of laser light, which depends on its photothermic effects, has been evaluated both in vitro and in vivo [13–21], but there are few studies reporting on the effect of laser light on fungal biofilms [22–24]. Our study evaluated the efficacy of two different laser wavelengths [an erbium:yttrium–aluminium–garnet (Er:YAG) laser with a wavelength of 2940 nm and diode laser with 810 nm wavelength] on different oral strains of *Candida albicans* grown in a 5-day biofilm.

Materials and methods

Yeast strains and growth conditions

Two clinical oral isolates of *Candida albicans* were used in the study. The first *C. albicans* strain, named SK1, was a clinical oral isolate from a patient suffering from a total denture stomatitis. The second *C. albicans* strain, named SK2, was a clinical oral isolate derived from an immunocompromised patient with oral mycosis. After 12 h of growth in glucose broth at 37°C, the *Candida* cells were harvested at the end of the logarithmic growth phase. Then, the yeast cells were washed three times with phosphate-buffered saline (PBS, pH 7.0) and standardised to 1×10^7 cells/ml.

Biofilm formation

The biofilm was established on the basis of Chandra et al. [39] and modified as follows: 100 μl of the standardised *C. albicans* cell suspension was put onto the surfaces of small discs placed in a 24-well tissue culture plate (Corning No 3524, Corning Inc., New York, USA). Either round glass slides (Menzel, Braunschweig, Germany), 12 mm in diameter, or machined titanium devices of the same diameter (Friadent, Mannheim, Germany) were used, covered with foetal calf serum (Biochrom, Berlin, Germany) for 24 h before the *Candida* cells were allowed to adhere for 90 min at 37°C (adhesion phase). After that time, non-adherent cells were removed from the slips by being gently washed with 2 ml PBS. The discs were then submerged in 2 ml of brain heart infusion broth (Oxoid, Wesel, Germany) and incubated for 5 days at 37°C. This medium was replaced every 24 h by the same new medium. Discs with no cells on their surfaces were treated in the same way and were used as negative controls. Control and experimental slips were incubated at 37°C for 5 days (biofilm growth phase).

Quantitative measurement of the biofilms

The biofilm mass was measured according to the method of Chandra et al. with a colorimetric assay that determines mitochondrial dehydrogenase activity, an indicator of the metabolic state of the fungal cells. This assay is based on the metabolic reduction of 2, 3-bis (2-methoxy-4-nitro-5-sulphophenyl)-5-((phenyl amino) carbonyl)-2H-tetrazolium hydroxide (XTT) to a water-soluble brown formazan product. For the quantitative measurement, the discs with biofilms were transferred to new 24-well tissue culture plates containing 2 ml PBS per well. To each well were added 25 μl XTT (1 mg/ml in PBS) and 2 μl menadione solution (1 mM in acetone). Plates were incubated at 37°C for 5 h. The entire contents of the well were transferred to a tube and centrifuged (5 min, 10,000 *g*). The amount of XTT–formazan in the supernatant was determined spectrophotometrically at 492 nm.

Exposure of the *Candida* biofilms to laser irradiation

The antimicrobial efficacies of two different laser wavelengths were studied. The parameters to be applied were chosen according to clinical evaluations [19, 23].

The Elexxion duros laser (Elexxion, Radolfzell, Germany) was employed, representing both an 810 nm wavelength diode laser and a 2940 nm wavelength Er:YAG laser.

1. Diode laser light of 810 nm wavelength was applied in slight contact mode with a 600 μm fibre in continuous wave mode (cw) at 1 W for 80 s. After each 20 s irradiation time a 30 s pause for cooling was included in the regimen [19]. The power density represented 353.7 W/cm^2.
2. Er:YAG laser light of 2940 nm wavelength was applied in pulsed mode (100 mJ, 10 Hz, 300 μs per

pulse), also for 80 s irradiation time. The 800 μm sapphire application tip was continuously cooled with sterile deionised water during the application of laser light and kept at a distance of 0.5 mm to 1 mm from the irradiated surface. According to the 13° divergence the energy density represented 12.0 J/ cm^2 and 15.2 J/ cm^2.

In order to test the efficacy of the laser irradiation regimen under conditions relevant for clinical situations, we irradiated the *Candida* biofilms for 80 s at room temperature. After removal of the growth medium, the discs were taken from the well plates and irradiated at the laser wave lengths described above. The treatments were performed by an oral surgeon conversant with laser application.

The irradiation time for both kinds of slides, for the diode as well as for the Er:YAG laser, added up to 80 s. According to the absorption spectrum of the diode laser, the glass slides were placed on a dark sterile background and were irradiated unilaterally. The titanium sleeves were irradiated from both sides. Irradiation was performed at least in duplicate at six different times.

Two glass slides and two titanium slides were left untreated and served as controls.

After treatment, irradiated and control slides were submerged in 2 ml PBS. No extra rinsing was performed.

The remaining *Candida* cells were then photometrically measured using the XTT-formazan method described above. Each test was performed at least in duplicate, and all values were obtained from sixfold application.

Scanning electron microscopy

Two more samples of the in vitro *Candida* biofilm on glass and titanium were fixed at the end of the laser application with freshly prepared paraformaldehyde (2% in PBS, Serva, Heidelberg, Germany), for at least 24 h at 8°C. The samples were dehydrated with ethanol (60–100%) and dried by the critical point method according to the instructions of the manufacturer (Polaron, Watford, UK). They were then sputtered with gold–palladium (Fisons Instruments, Uckfield, UK) prior to evaluation by scanning electron microscopy (SEM) (Zeiss DSM 960, Oberkochen, Germany) at 15 kV.

Each sample was qualitatively analysed to establish the number of *Candida* cells at the end of the laser procedure, their form, and the integrity of the fungal cells.

Statistical evaluation

Data were analysed with SAS 9.1 software (SAS Institute Inc., Cary, NC, USA). We used a heteroscedastic mixed linear model analysis (two-factor block design) using the SAS Procedure PROC MIXED with an analysis of variance–F statistic (ANOVA-F) approximation to examine the effect of the different treatments. Multiple comparisons with the control were performed, using the closure testing principle [25]. The results were regarded as significant if the P value was smaller than 0.05.

Results

Results of in vitro laser irradiation

There were no differences in the amount of cell growth between the glass and titanium surfaces colonised by the two isolates *C. alb.* SK1 and *C. alb.* SK2. No significant differences were observed between the different surfaces and with the two oral *Candida* isolates (*C. alb.* SK1, P= 0.4815; *C. alb.* SK2, P=0.3536) (Fig. 1).

The efficacy of the different treatments was similar for both surfaces, but the inter-treatment differences were significant for both *C. alb.* SK1 (P<0.0001) and *C. alb.* SK2 (P=0.0001).

The two lasers showed significant efficacy on vital *Candida* biofilms after the clinically relevant application time of 80 s compared to the controls as well as compared to each other.

(1) *C. albicans* SK1: control versus treatment by diode laser P<0.0001; control versus treatment by Er:YAG laser P<0.0001 (Table 1, Fig. 2).
(2) *C. albicans* SK2: control versus treatment by diode laser P<0.0001; control versus treatment by Er:YAG laser P<0.0001. Statistical significance was clearly observed for both oral *Candida* isolates (Table 1, Table 2 and Fig. 2).

Additionally, the statistical comparison of the diode laser versus Er:YAG laser revealed significant differences. The efficacy of the Er:YAG laser light exceeded that of the diode. The significant differences were obtained with each of the oral *Candida* isolates tested:

(1) *C. albicans* SK1: diode versus Er:YAG laser (P< 0.0059).
(2) *C. albicans* SK2: diode versus Er:YAG laser (P< 0.0001) (Tables 1 and 2; Fig. 2).

Scanning electron microscopy

Scanning electron microscopy was performed to visualise the efficacy of the two different laser wavelength compared to the controls. The complexity and the multilayer of the biofilms are clearly shown (Fig. 1). The efficacy of the diode laser light, applied in direct contact mode, is visualised in Fig. 3a and b. The cells seem to have been

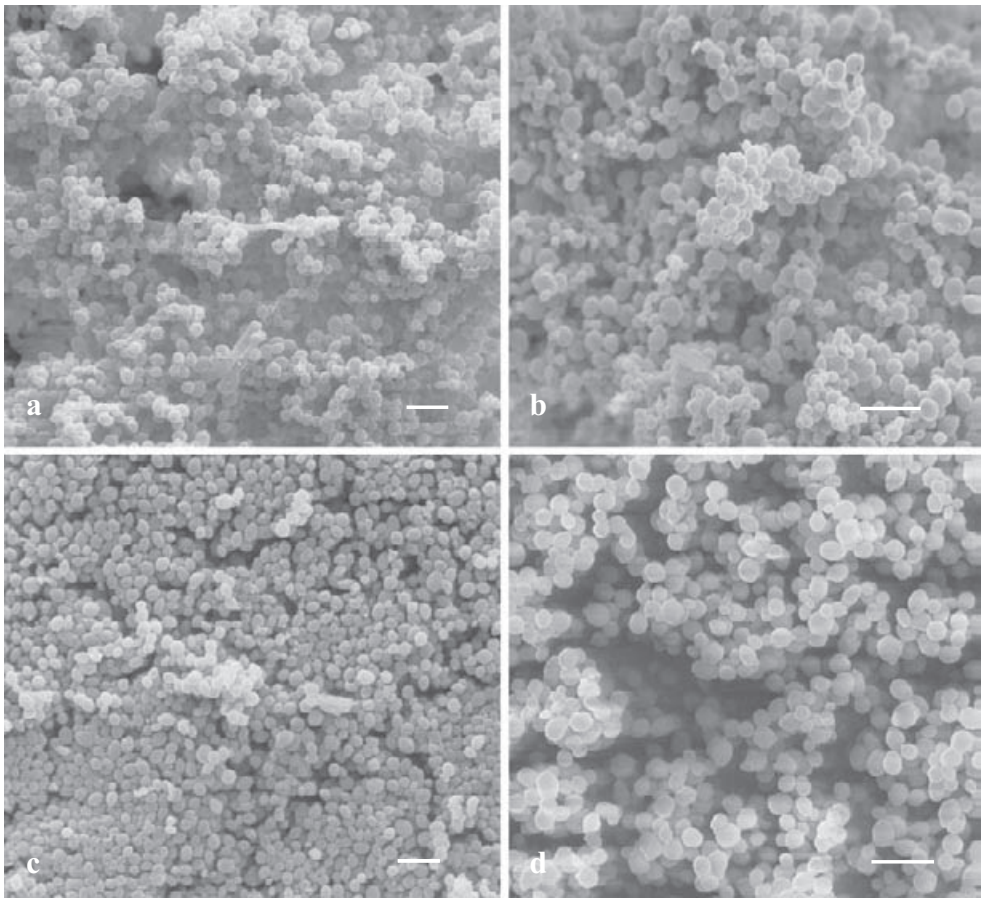

Fig. 1 SEM image of *Candida albicans* biofilms grown for 5 days on glass and titanium surfaces. No morphological difference between the two isolates *C. alb.* SK1 (**a**, **c**) and *C. alb.* SK2 (**b**, **d**) colonising glass (**a**, **b**) and titanium (**c**, **d**) surfaces became obvious in the SEM evaluation. Blastoconidia and pseudomycelia could be observed. All ×1,000. The *bars* represent 10 μm

squashed and melted by direct contact with the glass fibre of the diode laser. It is obvious that the fungal cells are left almost unchanged in the other areas. In contrast to these effects, the Er:YAG laser light, in combination with water cooling, has damaged the fungal cells to a greater extent, wherever it reached the surface, and removed nearly all damaged cells from the surface of the slides (Fig. 3c and d).

Discussion

Our investigation evaluated the efficacy of diode and Er:YAG laser light on *Candida albicans* biofilms. The basic objectives followed SEM observations of patients with failing dental implants. *Candida albicans* was seen as a frequent coloniser of infected peri-implant sites, in accordance with findings of other study groups [11]. Furthermore, it has been demonstrated that the biofilm network of microorganisms leads to significantly decreased levels of susceptibility to the conventional antimicrobial and antifungal agents [4]. In vitro biofilm models have been established on various surfaces to investigate different antimicrobial strategies with good reproducibility [26, 27]. In this study a biofilm model of *Candida*, based on the work of Chandra et al., was used and modified for the special investigational problems (see Methods).

Table 1 Evaluation of the laser treatment of *Candida albicans* SK1 on glass and titanium slides. The mean values (492 nm) of the photometric XTT measurement of untreated controls and treated samples after diode and Er:YAG laser irradiation are depicted in bold type. They were calculated from six repetitions. The standard error (*SE*) and minimum (*Min*) and maximum (*Max*) values are presented as well as the statistical analysis

Surface	Treatment	Mean value, 492 nm	SE	Min	Max	Statistical analysis	
Glass	Control	*0.25*	0.04	0.20	0.31	Control/diode, $P<0.0001$;	Diode/Er:YAG,
Glass	Diode	*0.03*	0.03	0.010	0.06	control/Er:YAG, $P<0.0001$	$P<0,0003$
Glass	Er:YAG	*0.01*	0.01	0.00	0.02		
Titanium	Control	*0.25*	0.05	0.18	0.30		
Titanium	Diode	*0.02*	0.03	0.00	0.06		
Titanium	Er:YAG	*0.00*	0.00	0.00	0.001		

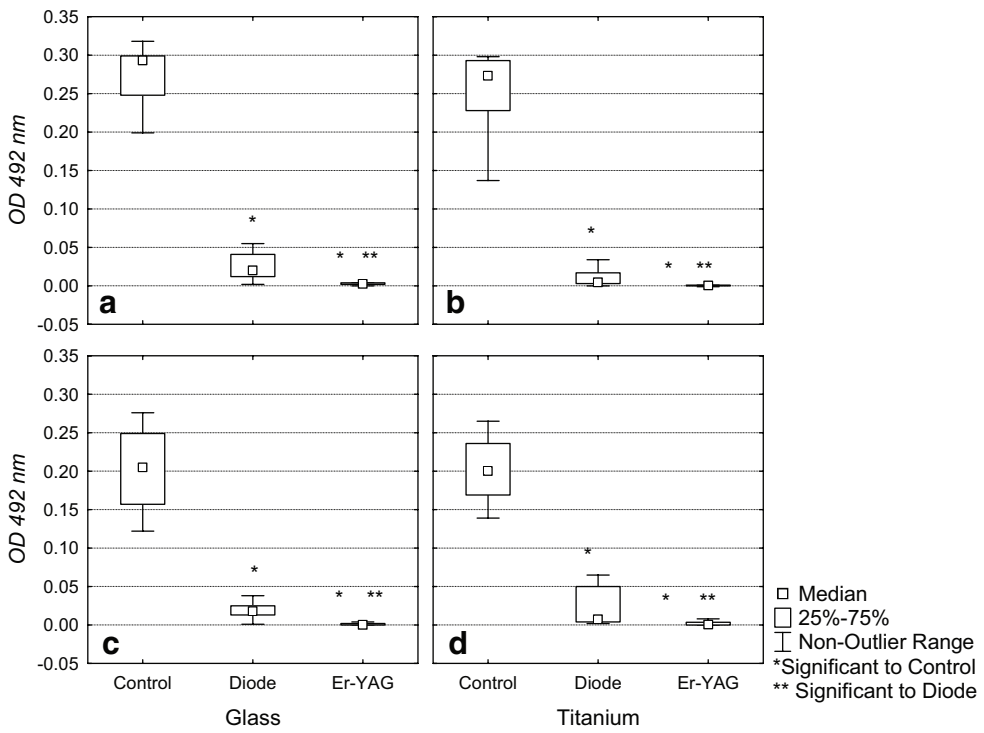

Fig. 2 Box plots of the efficacy of the treatment of the *Candida* biofilms by diode and Er:YAG irradiation on glass and titanium surfaces. The results of the evaluation on the oral isolates *C. albicans* SK1 (**a** and **b**) and *C. albicans* SK 2 (**c** and **d**) are depicted, in comparison with the control values. The trial was performed at least in duplicate with six repetitions. The absolute values (OD 492 nm) are depicted on the ordinates. The reduction in the viability of *Candida albicans* cells following the irradiation by diode and Er:YAG laser light for 80 s each is shown. Compared to the controls, the reduction with both laser regimens was statistically significant (*). A statistically significant difference was also demonstrated for the comparison of diode and Er:YAG laser (**). *OD* optical density

Even though the major role of periodonto-pathogens in the development of peri-implant infections has been scientifically confirmed, it has to be taken into account that the combination of bacteria and yeasts in biofilms results in an even higher pathogenic potential [28]. Particularly, elderly denture wearers show oral *Candida albicans* growth in frequencies above average [6, 7]. *C. albicans* as a commensal of normal oral flora can change into an opportunistic pathogen, when the immunological situation of the host changes, by expressing several pathogenicity factors [29]. These microorganisms are able to cause a variety of severe infections in immuno-deficiency situations [4, 5]. The peri-implant site next to rough implant surfaces reveals a decreased immune defence compared to the gingivo-periodontal situation [30, 31]. Therefore, *C. albicans* might find an optimal environment for its conversion into an opportunistic pathogen. In combination with the decreased susceptibilities of microorganisms towards conventional methods of treatment in the situation of biofilm formation, the evaluation of the efficacy of new methods, such as laser irradiation, is of scientific interest.

The efficacy of laser light of various wavelengths to decontaminate surfaces has been demonstrated repeatedly in vitro [14–17,32,33]. Its clinical use in the treatment of peri-implantitis has been described [19–21], but there are only a few studies on the direct effects of laser light on oral biofilms [34–37], and the reported efficacy of the laser treatment shows great variability. Additionally, different wavelengths have been used. Rovaldi and colleges [38], for example, found a 6-log bacterial decrease by photosensitising and following 662 nm laser irradiation in vitro. However, the same treatment mode applied to plaque

Table 2 Evaluation of the laser treatment of *Candida albicans* SK2. Treatment and experimental and analytical conditions as in Table 1. *SD* standard deviation

Surface	Treatment	Mean value, 492 nm	SD	Min	Max	Statistical analysis	
Glass	Control	*0.20*	0.05	0.15	0.25	Control/diode, $P<0.0001$;	Diode/Er:YAG,
Glass	Diode	*0.02*	0.01	0.01	0.03	control/Er:YAG, $P<0.0001$	$P<0.0001$
Glass	Er:YAG	*0.00*	0.00	0.00	0.003		
Titanium	Control	*0.23*	0.07	0.16	0.38		
Titanium	Diode	*0.02*	0.03	0.00	0.06		
Titanium	Er:YAG	*0.00*	0.01	0.00	0.02		

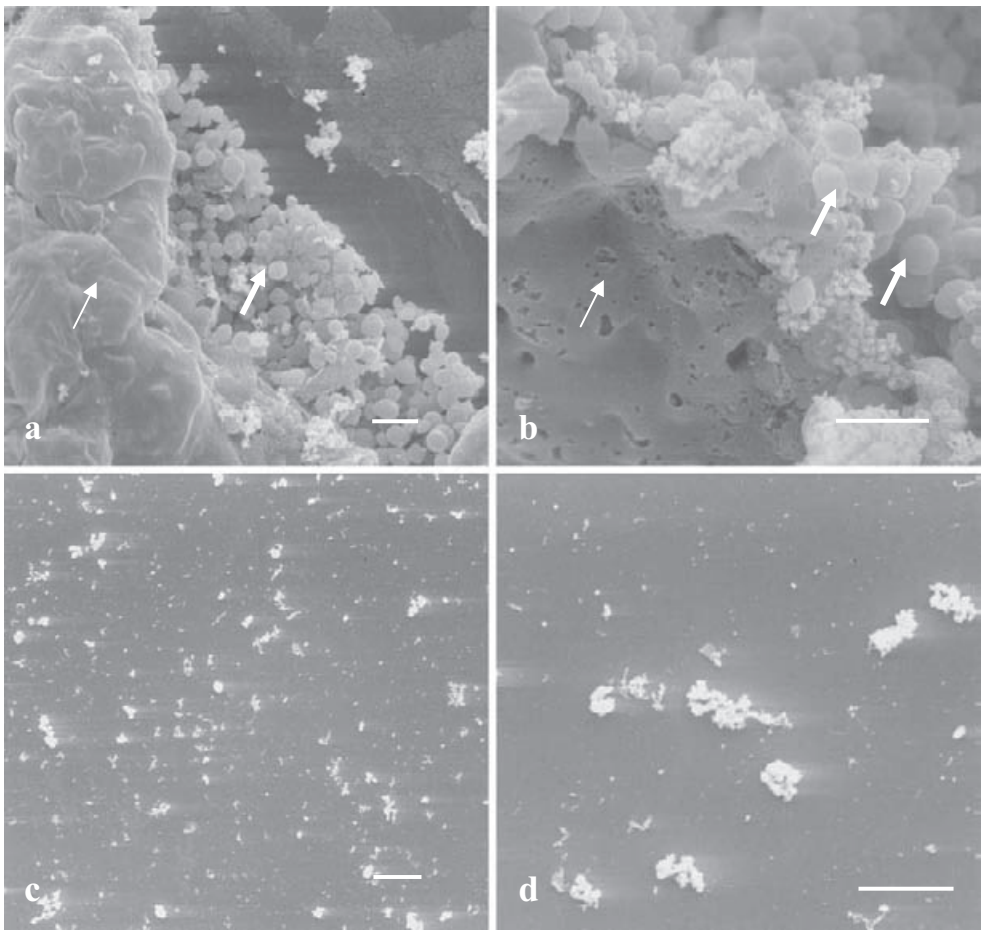

Fig. 3 Efficacy of the diode (**a**, **b**) and the Er:YAG (**c**, **d**) laser treatments demonstrated by SEM. **a**, **c** ×1,000; **b**, **d** ×3,000. The *bars* represent 10 μm. The *Candida* cells seem to be squashed and melted due to the direct contact with the laser fibre (*thin arrows*). In other areas the fungal cells are left almost unchanged (*thick arrows*). In combination with the cooling water, this Er:YAG laser treatment damaged the fungal cells to a greater extent wherever it reached the surface and nearly removed the damaged cells from the surface of the slides

bacterial biofilm samples from periodontally affected persons by other authors only led to a 75–92% reduction, which means a ≤ 2-log decrease [17]. Schwarz and colleagues found a high efficacy of Er:YAG laser irradiation on intra-orally grown early biofilms [18]. This group has shown that the efficacy of the Er:YAG wavelength increased above the effects of conventional treatment. The results of our study support the findings of Schwarz and co-workers and, furthermore, show the efficacy of the Er:YAG laser on mature *Candida* biofilms.

There are, likewise, only a few studies evaluating the effect of laser light on fungal biofilms [22–24]. Ward et al. determined laser light of 1,064 nm wavelength, applied at 10 J, 8 ms, 10 Hz, to be effective on different bacteria and yeasts on agar plates, without changing the surface of the agar in these power settings [24]. Donnelly and co-workers used specific staining methods to increase the efficacy of laser irradiation on *C. albicans* biofilms on the oral mucosa to evaluate the effect of photodynamic antimicrobial therapy (PDT) on both planktonic- and biofilm-grown *Candida albicans* cells [22]. They found it necessary to increase photosensitiser concentration and incubation time, as well as laser light doses, over clinically capable measures to achieve high decontamination rates for *Candida* grown in biofilms compared to the planktonic form. The study group around De Souza [23] aimed at the effects of low-level diode laser radiation (685 nm) associated with photosensitisers on the viability of different species of *Candida* genus. Laser radiation in the presence of methylene blue reduced the number of colony forming units per millilitre by 88.6% for *C. albicans*. Though the PDT mode of antibacterial operation differs from that of high-power laser light, the results of our study considerably exceeded those low-level therapy results.

Conclusion

Candida albicans biofilms play a major role on mucosal surfaces and different medical devices where the immunological defence is diminished. The accumulation of the microorganisms in biofilms decreases their susceptibilities towards conventional treatment modalities. Our study was able to show the efficacy of diode light, and particularly Er:YAG laser light, on *Candida albicans* biofilms grown on glass and titanium surfaces after a clinically tenable

application time. Especially, the treatment outcome on titanium surfaces makes the results valuable for laser application in the treatment of peri-implant infections. When diode laser light is applied on dental implants, adequate cooling-off times will be essential, to avoid overheating of the adjacent bone.

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Entfernung bakterieller Plaque-Biofilme von strukturierten Titanimplantaten unter Verwendung von Laserwellenlängen im Bereich von 3 µm

Frank Schwarz, Daniel Ferrari, Christian Popovski, Jürgen Becker

Schlüsselwörter

Biofilm Modell, Periimplantäre Infektionen, Initialtherapie, Biokompatibilität

Zusammenfassung

Das Ziel des vorliegenden Übersichtsartikels ist es, auf Grundlage derzeitiger Evidenz, die Entfernung bakterieller Plaque-Biofilme von strukturierten Titanimplantaten unter Verwendung von Laserwellenlängen im Bereich von 3 µm zu bewerten.

Dieser Artikel zeigt Auszüge aus dem Buch "Periimplantäre Entzündungen – Ätiologie, Pathogenese, Diagnostik und aktuelle Therapiekonzepte" von Frank Schwarz und Jürgen Becker, erschienen im Quintessenz Verlag, Berlin 2006.

Einleitung

In den letzten Jahren konnte in einer Vielzahl sowohl tierexperimenteller als auch klinischer Untersuchungen die Akkumulation bakterieller Biofilme als primärer ätiologischer Faktor für die Entstehung und Progression periimplantärer Entzündungen definiert werden[1-3]. Weiterhin können zahlreiche Risikofaktoren additiv wirksam werden und den Verlauf der Erkrankung negativ beeinflussen. Das Vorhandensein eines oder mehrerer dieser Faktoren kann im Rahmen einer Spätkomplikation nach entzündlicher Veränderung der periimplantären Gewebestrukturen zum Implantatverlust führen.

Um einer Progression der Erkrankung entgegenzuwirken, muss durch eine kausal gerichtete Therapie primär versucht werden, die pathogene Mikroflora zu reduzieren[4]. Die Entfernung subgingivaler Konkremente sowie des bakteriellen Biofilms von Titanimplantaten wird jedoch durch verschiedenste Implantatoberflächenmodifikationen erschwert[5].

Entstehung und Wachstum oraler Plaque-Biofilme

Unter einem Biofilm versteht man eine mikrobielle Ansiedelung auf Oberflächen jeglicher Art. Es handelt sich dabei um räumlich organisierte Gemeinschaften von Mikroorganismen, welche mit einer Oberfläche verbunden und in einer extrazellulären Matrix eingebettet sind[6]. Nach der Organisation innerhalb des Biofilms, können sich die Bakterien wie in einem mehrzelligen Organismus verhalten. Hierzu gehören nachfolgende Merkmale:

– Ausbildung einer protektiv wirksamen Glycocalyx in Form einer extrazellulären Matrix
– veränderte metabolische Aktivität mit schnellerer Erholung von Hungerphasen
– Mikrokolonien mit funktioneller Heterogenität

Durch diese spezifischen Verhaltensweisen kann die Überlebensfähigkeit der Mikroorganismen selbst in einem ungünstigen Milieu ermöglicht werden. Die Oberfläche eines Biofilms ist weiterhin in der Lage bestimmte Stoffe freizusetzen, welche bakterizid wirksame Substanzen, Antibiotika sowie Angriffe der Immunabwehr weitestgehend neutralisieren können. Obwohl von den Mikroorganismen im Biofilm kontinuierlich Antigene freigesetzt werden, welche wiederum die Produktion spezifischer Antikörper auslösen, ist es den Phagozyten nicht möglich die Glycocalyx zu penetrieren. Innerhalb von Minuten bis Stunden bildet sich auf einer Implantatoberfläche durch selektive Adsorption eine organische Ablagerung aus Glykoproteinen des Speichels. Dieses sogenannte erworbene Pellikel enthält Anteile an hochmolekularem Mucin, α-Amylase sowie Prolin-reichen Glykoproteinen[7]. Bei der initialen Biofilmentstehung auf Titanimplantaten scheint die Adsorption des Speichelproteins Albumin insbesondere in Gegenwart von freien Ca_2^+-Ionen eine besondere Rolle zu spielen[8].

Die Adhäsion von Mikroorganismen an Oberflächen in einem wässrigen Milieu wurde in vier Stufen beschrieben[9]:

Phase 1: Initialer Transport der Mikroorganismen zur Oberfläche durch Sedimentation, Flüssigkeitsbewegung oder aktive Fortbewegung.

Phase 2: Initiale, noch reversible Adhäsion über van der Waals'sche Bindungskräfte oder Elektrostatische Anziehung.

Phase 3: Attachment der Mikroorganismen und feste, irreversible Verbindung zur Oberfläche über kovalente, ionische oder Wasserstoffbrückenbindungen.

Phase 4: Kolonisation und Ausbildung eines Biofilms.

Neben Streptokokken nehmen insbesondere Fusobakterium-Arten (v. a. *F. nucleatum*) beim Plaquewachstum eine besondere Bedeutung ein. Sie besitzen die Fähigkeit an sämtliche bisher bekannte orale Mikroorganismen zu binden (Koaggregation), ohne jedoch eine Koadhäsion untereinander eingehen zu können. Experimentelle Untersuchungen konnten jedoch nachweisen, dass mit Albumin oder Speichel beschichtete Titanoberflächen im Vergleich zur Schmelzoberfläche eine signifikant veränderte initiale Adhäsion spezifischer Mikroorganismen zeigten[7,10,11].

Die individuelle Plaquebildungsrate sowie Lokalisation und mikrobielle Zusammensetzung ist von vielen Faktoren abhängig[6]. Hierzu zählen u. a. die Verfügbarkeit erforderlicher Nährstoffe, die Fließrate, Viskosität und Zusammensetzung des Speichels sowie allgemeine Faktoren wie das Alter oder das Vorhandensein systemischer Erkrankungen. Mit zunehmendem Wachstum der Mikroorganismen entstehen zudem sauerstoffarme Zonen innerhalb des Biofilms, welche zu einer mengenmäßigen Zunahme anaerober Bakterien wie z. B. *Veillonella* oder *Actinomyces* spp. führen kann. Weiterhin wird die individuelle Plaquebildungsrate auch durch das Vorhandensein mechanischer Retentionsstellen sowie eine schlechte Mundhygiene gefördert. Bei der Entstehung periimplantärer Infektionen kommt insbesondere der oralen Biofilmbildung in Abhängigkeit von der Oberflächenrauhigkeit der Implantatoberfläche eine übergeordnete Bedeutung zu. Grundsätzlich konnten auf Grundlage experimenteller sowie klinischer Studien nachfolgende Erkenntnisse gewonnen werden[12]:

– Raue Oberflächen von Kronen, Implantatabutments oder Prothesenbasen akkumulieren und bewahren signifikant mehr Plaque-Biofilme als glatte Oberflächen.
– Nach einer ungestörten Plaquebildung über mehrere Tage zeigen raue Oberflächen einen qualitativ reiferen Plaque-Biofilm durch ein überproportional hohes Vorkommen beweglicher Mikroorganismen und Spirochäten.
– Es besteht eine direkte Korrelation zwischen der Oberflächenrauhigkeit und den klinischen Entzündungsparametern im Bereich des marginalen Parodontiums.

Grundsätzlich ist auf allen derzeit verfügbaren Titanoberflächen ein makroskopisch sichtbarer initialer Biofilm (Anfärbung mit Erythrosin) innerhalb von 12 bis 48 Stunden vorhanden[13]. Die geringste Plaqueakkumulation zeigen hierbei polierte Implantatoberflächen (Ra = 0,03 µm), sandgestrahlte und säuregeätzte Implantatoberflächen (Ra = 1,24 µm) dagegen die größte. Bei der Bewertung dieser Ergebnisse ist jedoch darauf zu achten, dass es sich um supragingivale Biofilme handelt und eine direkte Übertragung auf die subgingivale Region nicht zulässig ist. Grundsätzlich kann jedoch davon ausgegangen werden, dass die subginigvale Biofilmformation eine vergleichbare Abhängigkeit von der Oberflächenrauigkeit aufweist.

Im subgingivalen Bereich herrschen überwiegend anaerobe Bedingungen vor. Bei den subgingivalen Plaque-Biofilmen unterscheidet man zwischen adhärenter und nicht adhärenter Plaque („schwimmende Plaque"). Die adhärente subgingivale Plaque entspricht in ihrem Aufbau prinzipiell der supragingivalen Plaque. Die mikrobielle Zusammensetzung der subgingivalen Plaque zeigt bei

einer manifesten Gingivitis ca. 25 % Streptokokken, 25 % *Actinomyces* spp., 25 % gramnegative Stäbchen und 25 % anderer Bakterienarten mit einem nur geringen Anteil an Spirochäten. Die Zusammensetzung verschiebt sich bei einer marginalen Parodontitis zu einem mengenmäßigen Anteil anaerober Bakterien von bis zu 90 %. Hierzu gehören zu ca. 75 % gramnegative Mikroorganismen. Während bei manifesten Gingivitiden überwiegend die anaeroben *Fusobacterium* spp. dominieren, sind bei marginalen Parodontopathien der anaerobe *P. gingivalis* und der kapnophile *A. actinomycetemcomitans* involviert[6].

Vorherrschend waren Gram-negative anaerobe sowie fakultativ anaerobe Bakterien[14,15].

Durch eine weitere Mineralisierung des oralen Plaque-Biofilms entsteht Zahnstein. Dieser ist grundsätzlich immer mit einer Schicht nicht kalzifizierter Plaque bedeckt. Die Mineralisation dauert hierbei Monate bis Jahre (Abb. 1).

Es können vier verschiedene Kalziumphosphatkristallite unterschieden werden:

– $CaH(PO_4)$ x $2H_2O$ = Dikalziumphosphat-dihydrat (Brushit)
– $Ca_8(PO_4)_4(HPO_4)_2$ x $5H_2O$ = Oktakalziumphosphat – v. a. supragingival in äußeren Lagen
– $Ca10(PO_4)_6(OH)_2$ = Hydroxylapatit – v. a. supragingival in inneren Lagen
– $[Ca_3(PO_4)_2]_3$ x H_2O = Trikalziumphosphat (Whitlockite) – v. a. subgingival

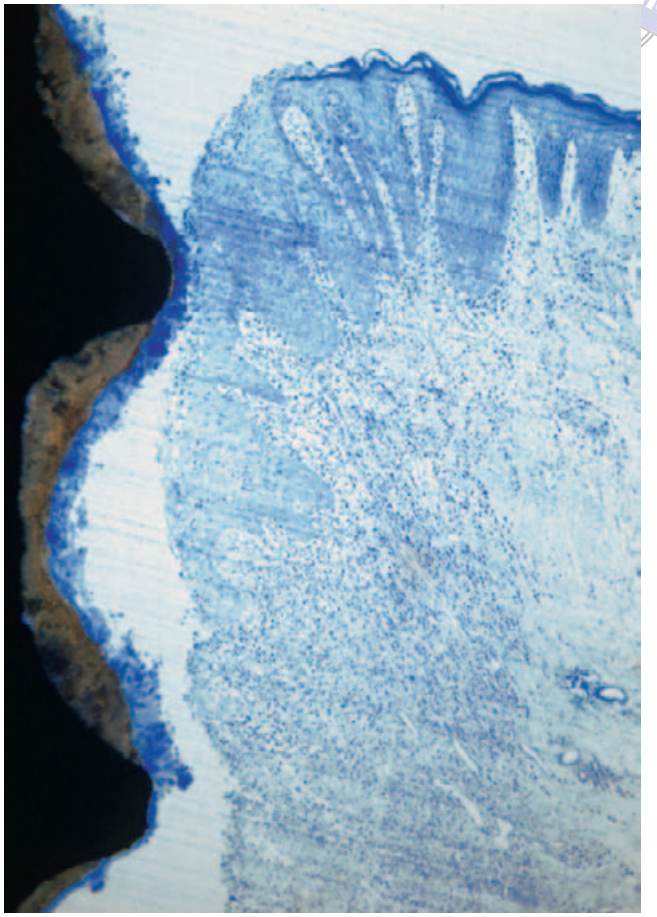

Abb. 1 *Histologische Darstellung einer supra- und subgingivalen Ausbildung mineralisierter Plaque-Biofilme mit sekundärer Infiltration des angrenzenden periimplantären Gewebes (Hundemodell, Toluidinblau, Vergr. 200-fach).*

Mechanische Therpieansätze

Zur supra- und subgingivalen Belagentfernung stehen heutzutage Polierbürsten, Gummipolierer, Teflon-, Kunststoff-, Karbon- oder Titankürretten, speziell modifizierte Arbeitsenden für Ultraschallsysteme sowie Pulverstrahlgeräte zur Verfügung[16-18]. Grundsätzlich sollten Titanimplantatoberflächen jedoch nur unter Verwendung solcher Therapieinstrumente gereinigt werden, welche eine geringere Härte als Titan selbst aufweisen.

Als Nachteile dieser Therapieinstrumente sind jedoch die nur unzureichende Reinigungsmöglichkeit der texturierten Implantatoberfläche sowie beim Einsatz von Pulverstrahlgeräten – trotz guter Reinigungsleistung – die Gefahr einer Emphysembildung zu nennen[16,18-20].

Als weiterer Nachteil konventioneller Ultraschallsysteme ist neben der Hitzeentwicklung an der Arbeitsspitze bei unzureichender Kühlung[21], die bei der Behandlung auftretende Aerosolbildung kritisch zu bewerten[22]. Weiterhin kann die überwiegend horizontal gerichtete Schwingung des Arbeitsendes einen Verlust der noch vorhandenen Restosseointegration verursachen. Demnach ist eine Verwendung konventioneller Ultraschallsysteme nur bei einer verbleibenden Restosseointegration von mindestens 50 % der Implantatlänge zu empfehlen. Um einige dieser Probleme zu umgehen, wurde kürzlich ein modifiziertes Ultraschallsystem (Vector® System, Dürr, Bietigheim Bissingen, Deutschland) (VUS) entwickelt, mit welchem eine vertikale Schwingung des Arbeitsendes bei einer Frequenz von 25 kHz möglich ist. In einer ersten klinischen Untersuchung wurden bei der nichtchirurgischen Parodontaltherapie nach sechs Monaten vergleichbare Attachmentgewinne wie nach handinstrumentellem Scaling und Wurzelglätten erzielt[23]. Die Effizienz bei der Entfernung subgingivaler Konkremente von parodontal erkrankten Wurzeloberflächen war jedoch deutlich reduziert[24]. Zur Entfernung bakterieller Plaque-Biofilme von Implantatoberflächen wird neben diesen konventionellen Therapieansätzen neuerdings auch der Einsatz von Laserwellenlängen im Bereich von 3 µm empfohlen.

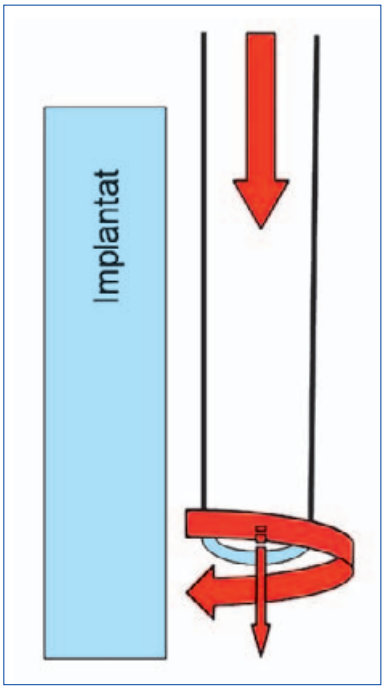

Abb. 2a *Schematische Darstellung des axialen und radialen Strahlungsmusters am Austrittspunkt einer Kegelstumpffaser (KaVo, Biberach, Deutschland). Insbesondere die radiale Komponente kann klinisch zu einer Perforation im Bereich der periimplantären Mukosa führen.*

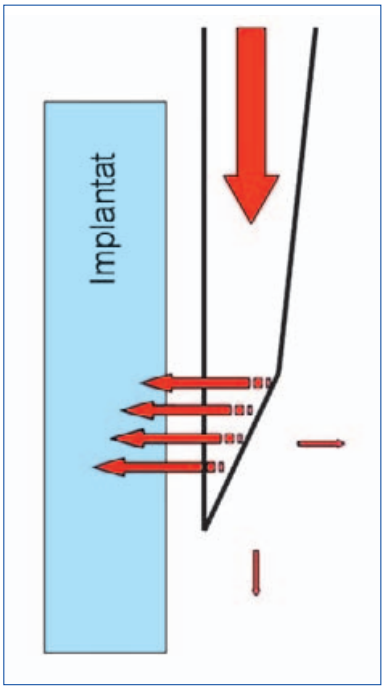

Abb. 2b *Modifizierte Faser mit einseitiger sowie flächiger Abstrahlung über die gesamte Faserlänge (elexxion, Radolfzell, Deutschland).*

Charakteristika des Er:YAG- und Er,Cr:YSGG-Lasers

Der Er:YAG-Laser (ERL) wurde im Jahr 1974 von Zharikov et al.[25] als Festkörperlaser mit einer Wellenlänge von 2,940 nm im nahen bis mittleren Infrarotbereich vorgestellt. Die Besonderheit dieser Wellenlänge liegt in der Tatsache, dass die charakteristische Absorption des ERL in Wasser ungefähr 15-mal größer als die des CO_2-Lasers und sogar 20.000-mal größer als die des Nd:YAG-Lasers ist[26,27]. Bei der sogenannten „thermomechanischen Ablation" beruht der Abtrag von biologischem Gewebe in erster Linie darauf, dass der Anteil des in ihm enthaltenen Wassers bei Absorption von kurzen Laserpulsen einen sprungartigen Übergang vom flüssigen in den gasförmigen Aggregatzustand erfährt. Begleitet durch die schnelle Expansion des Wassers entsteht hierbei kurzzeitig ein genügend hoher Druck um Gewebesubstanz in gewünschter Weise abzutragen[28,29]. Die zur Ablation benötigte Energie wird demnach nicht von der Verdampfungswärme der höherschmelzenden Gewebesubstanz bestimmt, sondern durch die wesentlich niedriger liegende Verdampfungswärme des Wassers. Neben Wasser weisen insbesondere auch OH-Gruppen als Bestandteil von Hydroxylapatit eine relativ hohe Absorption im Bereich von 2,940 nm auf, obwohl sich das Maximum hier im Bereich von rund 2,800 nm befindet[30]. Durch den zusätzlichen Einsatz einer Wasserkühlung lässt sich eine Überwärmung des Gewebes zum einen durch direkte Kühlwirkung und zum anderen durch eine Absorption übermäßiger Laserenergie weiter reduzieren[31]. In einigen tierexperimentellen Untersuchungen wurde die Knochenheilung nach Osteotomie mit einem ERL histologisch untersucht[32–34]. Hierbei konnte beobachtet werden, dass eine effektive Knochenabtragung möglich ist, ohne ausgedehnte thermische Veränderungen im angrenzenden Gewebe zu verursachen[33]. Im Vergleich zur konventionellen Fräse war die Knochenheilung nach Bestrahlung mit einem ERL sogar verbessert[35]. Auch die Osseointegration enossaler Titanimplantate verlief vergleichbar oder sogar besser als nach konventioneller Implantatbettpräparation[36,37]. Derzeit wird vermutet, dass der Laser einen biostimulatorischen Effekt auf den Knochen ausüben könnte, welcher zu einer verbesserten Wundheilung führt. Bevor der ERL zur Osteotomie empfohlen werden kann, sind jedoch weitere Untersuchungen notwendig.

Die Er,Cr:YSGG-Laser (Erbium, Chromium-doped: Yttrium-Scandium-Gallium-Garnet) (ERCL) mit einer Wellenlänge von 2,780 nm sowie Er:YSGG-Laser (Erbium-doped: Yttrium-Scandium-Gallium-Garnet) mit einer Wellenlänge von 2,790 nm weisen dagegen eine höhere Absorption in OH-Ionen auf als in Wasser[30]. Die ausgezeichnete Absorp-

tion dieser Wellenlängen sowohl im Weich- als auch Hartgewebe führten in den vergangenen Jahren zu einem gesteigerten Interesse diese Systeme zur Behandlung periimplantärer Infektionen. Klinische Untersuchungen liegen bisher jedoch nur für den ERL mit einer Wellenlänge von 2,940 nm vor[38–40]. Die hohe Absorption der hier vorgestellten Wellenlängen sowohl in Wasser als auch OH-Ionen ermöglichen auch eine Entfernung bakterieller Plaque-Biofilme.

Entfernung bakterieller Plaque-Biofilme – Er:YAG-Laser

Vorhergehende *In-vitro*-Untersuchungen zeigten, dass eine Entfernung subgingivaler Konkremente von parodontal erkrankten Wurzeloberflächen mit dem ERL ab einer Energiedichte von 10,6 J/cm^2 möglich ist[24].

Um auch eine nichtchirurgische Therapie periimplantärer Infektionen an schraubenförmigen Titanimplantaten zu ermöglichen, wurde für den ERL eine spezielle kegelstumpfförmige Faser mit axialem und radialem Strahlungsmuster entwickelt (KaVo, Biberach, Deutschland) (Abb. 2a). Als potenzieller Nachteil der radialen Strahlungskomponente muss, insbesondere bei dünnem Gingivatyp, die Gefahr einer Perforation im Bereich der vestibulären Mukosa genannt werden. Trotz komplikationsloser Abheilung kann dies mit einem erhöhten Rezessionsanstieg und somit ästhetischen Nachteilen verbunden sein[38]. Diese Nachteile können durch einen einseitig, zur Implantatoberfläche gerichteten Strahlungsverlauf (elexxion, Radolfzell, Deutschland) vermieden werden (Abb. 2b). Diese neu entwickelte modifizierte Faser ermöglicht daneben eine flächige Abstrahlung über die gesamte Faserlänge, was in ersten klinischen Versuchen zu einer optimierten Effizienz insbesondere bei der nichtchirurgischen Therapie periimplantärer Infektionen führte (Studie in der Auswertung) (Abb. 3).

Erste klinische Fallberichte weisen auch auf ein Potenzial des ERL zur effektiven Entfernung bakterieller Biofilme von Titanimplantaten hin[41–43]. Hierbei wurden sechs von insgesamt acht nicht erhaltungswürdigen Implantaten (TPS) vor der Explantation mit einem ERL bei einer Energieeinstellung von 100 mJ und 10 Hz bestrahlt (12,7 J/cm^2). Die Auswertung erfolgte anhand rasterelektronenmikroskopischer Aufnahmen. Auf beiden Implantaten der Kontrollgruppe waren flächenhafte Konkrementablagerungen bis auf Höhe der ehemaligen Restosseointegrationsgrenze erkennbar. Im Gegensatz hierzu waren fünf Implantate der Testgruppe weitestgehend frei von Konkrementen. Es zeigten sich jedoch kleine Areale residualer Auflagerungen ins-

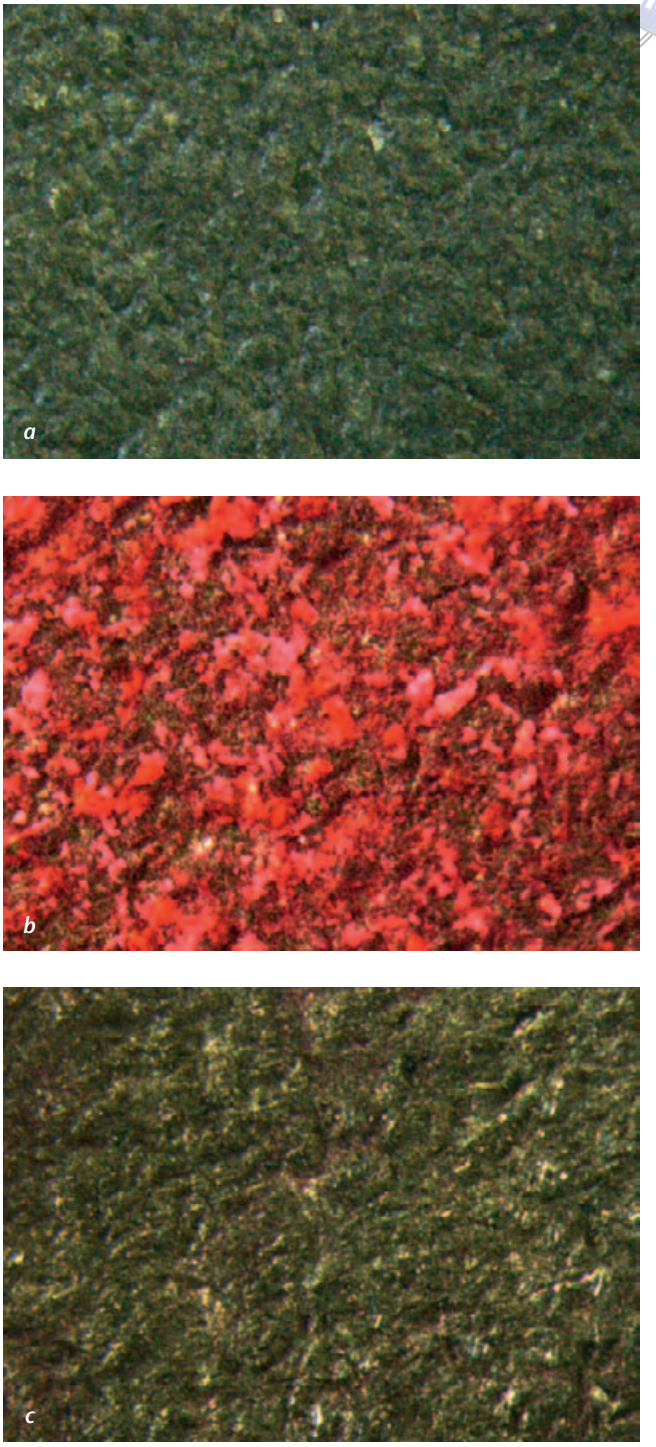

Abb. 3a–c *Sterile sandgestrahlte und säuregeätzte Titanimplantatoberfläche (a). Homogener Plaque-Biofilm nach 48 Stunden Tragezeit in einem intraoralen Splintsystem (b). Nahezu vollständige Entfernung der Plaque-Biofilm Areale nach Bestrahlung (Faser aus Abb. 2b) (100 mJ/ 10 Hz) mit einem Er:YAG Laser (elexxion delos, Radolfzell).*

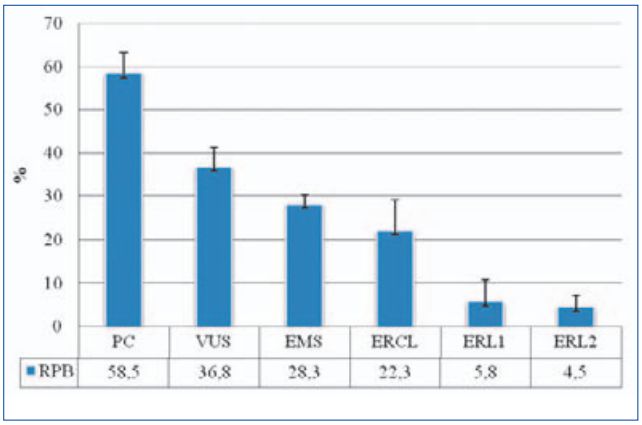

Abb. 4 *Vergleichende Darstellung Residualer Plaque-Biofilm Areale (RPB) auf strukturierten (SLA) Titanoberflächen nach Therapie[13,42,49].*
PC: Kunststoffküretten + lokale Spülung mittels Chlorhexidindiglukonat (CHX)
VUS: Vector®-Ultraschallsystem + Polyether-Etherketon (PEEK) Faser
EMS: Piezon Master 600® (EMS, Nyon, Switzerland) + PEEK Faser + Spülung mittels Chlorhexidindiglukonat
ERCL: Er,Cr:YSGG-Laser (2,0 W, 25 Hz, Waterlase® MD, Biolase)
ERL1: Er:YAG-Laser (12,7 J/cm², KEY® 3, KaVo, Biberach)
ERL2: Er:YAG-Laser (10–12 J/cm², delos®, Elexxion, Radolfzell)

besondere im Bereich der Gewindegänge von Schraubenimplantaten. Auf einem Hohlzylinderimplantat waren gar flächenhafte Konkrementablagerungen nachweisbar. Dagegen ließen sich keine thermischen Veränderungen der Oberflächenstruktur wie Aufschmelzungen oder Kraterbildungen nach Bestrahlung mit dem ERL nachweisen[41]. Neben der Entfernung des Biofilms von der Implantatoberfläche kommt auch der Enukleation des Granulationsgewebes aus dem periimplantären Knochendefekt eine große therapeutische Bedeutung zu. Die Ergebnisse einer klinischen Untersuchung konnten zeigen, dass im Rahmen der chirurgischen Parodontaltherapie eine suffiziente Ablation des Granulationsgewebes aus intraossären Defekten mit einem ERL möglich ist[44].

Ein erster Vergleich der Effektivität eines ERL mit der des VUS und Kunststoffküretten in Kombination mit lokaler Chlorhexidindiglukonat-Spülung (PC) bei der Entfernung bakterieller Plaque-Biofilme von SLA-Implantatoberflächen bestätigte diese Beobachtungen. Die morphometrische Bestimmung der prozentualen Verteilung residualer Plaqueareale (%) nach Instrumentierung ergab folgende Werte: PC (61,08 ± 11,15) > VUS (36,79 ± 4,46; P < 0,001) > ERL (5,78 ± 5,13; P < 0,001). Mit keiner dieser Therapieansätze war es jedoch möglich die Biokompatibilität der kontaminierten Implantatoberflächen im Vergleich zur nicht kontaminierten sowie unbehandelten Kontrollgruppe wieder herzustellen[42].

Erste mikromorphologische Veränderungen nach Bestrahlung mit einem ERL (Fokusabstand: 20 mm) konnten bei SLA sowie Titan-Plasma-Beschichtungen (TPS) bereits ab einer Energiedichte von 7 Jcm² beobachtet werden[45]. Unter Verwendung einer kegelstumpfförmigen Faserspitze wurden bei TPS-Oberflächen erste thermische Schäden jedoch erst ab Energiedichten von 8,9 Jcm² festgestellt[46]. Die Führung der Faser erfolgte hierbei im kontaktlosen Modus ohne Wasserkühlung in einem Anstellwinkel von 90° zur Implantatoberfläche. Dagegen zeigten SLA-Oberflächen erste thermische Veränderungen bei Energiedichten von 11,2 Jcm², mit Hydroxylapatit beschichtete Implantate (HA) bei Energiedichten von 17,8 Jcm² und strukturpolierte Implantatoberflächen (MP) bei Energiedichten von 28 Jcm² [46]. Unter Verwendung einer Wasserkühlung und parallelen Führung der Faser in Kontakt zur Implantatoberfläche konnte eine Instrumentierung von SLA-, TPS-, HA- und MP-Oberflächen bei Energiedichten von 12,7 Jcm² durchgeführt werden. Morphologie und Biokompatibilität der bestrahlten Implantatoberflächen waren im Vergleich zur unbehandelten Kontrollgruppe nicht verändert[47]. Bei einer kontinuierlichen Bestrahlungsdauer von 120 Sekunden (540 µm Faser, non-contact, 120 mJ, 10 Hz) wurde die kritische Temperaturschwelle am Knochen-Implant-Interface (SLA, TPS, HA Implantate) von 47°C unter experimentellen Bedingungen nicht erreicht[48].

Entfernung bakterieller Plaque-Biofilme – Er,Cr:YSGG-Laser

Erste experimentelle Untersuchungen ergaben, dass auch durch den Einsatz eines ERCL der Anteil residualer Biofilme auf strukturierten Implantatoberflächen in Abhängigkeit von der Energieeinstellung signifikant reduziert werden kann. So führte eine Bestrahlung (25 Hz) von SLA Implantatoberflächen zu nachfolgenden Ergebnissen: 53,8 ± 2,2 (0,5 W); 49,3 ± 5,8 (1,0 W); 29,3 ± 7,5 (1,5 W); 22,3 ± 6,8 (2,0 W); 9,8 ± 6,2 (2,5 W). Die Biokompatibilität der Titanoberflächen konnte jedoch im Vergleich zur unbehandelten Kontrollgruppe nicht wieder hergestellt werden[49]. Eine Bestrahlung von SLA Implantatoberflächen konnte bis zu einer Energie von 2 Watt (25 Hz) ohne Aufschmelzungen oder strukturelle Veränderungen durchgeführt werden[49] (Abb. 4).

Fazit für die Praxis

Die vergleichende Darstellung und Bewertung derzeit verfügbarer Untersuchungen zur Entfernung bakterieller

Plaque-Biofilme von strukturierten Titanimplantatoberflächen deuten darauf hin, dass sowohl Er:YAG- als auch Er,Cr:YSGG-Laser einen deutlichen Vorteil gegenüber den konventionell verfügbaren Therapiemethoden bieten. Dieser Vorteil zeigt sich insbesondere in der Möglichkeit der homogenen und vollständigen Entfernbarkeit sowohl nicht mineralisierter als auch mineralisierter (Er:YAG-Laser) Biofilme.

Diese Ergebnisse werden insbesondere beim nächsten Generationswechsel von mikro- zu nanostrukturierten Titanimplantatoberflächen weiter an klinischer Relevanz gewinnen.

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Removal of bacterial plaque biofolms from structured titanium implant surfaces using laser wavelengths within the range of 3 µm.

Key words: Biofilm model, peri-implant infections, initial therapy, biocompatibility

Summary

The aim of the present review paper is to evaluate, based on the currently available evidence, the removal of bacterial plaque biofilms from structured titanium implant surfaces using laser wavelengths within the range of 3 µm.

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REVIEW ARTICLE

Laser applications in oral surgery and implant dentistry

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Abstract Lasers have been used for many years in oral surgery and implant dentistry. In some indications, laser treatment has become state of the art as compared to conventional techniques. This article is a comprehensive review of new laser applications in oral surgery and implant dentistry. One of the most interesting developments over the last years was the introduction of the 9.6-μm CO_2 laser. It has been shown in the recent literature that the use of this new device can preserve tissue with almost no adverse effects at the light microscopic level. In contrast, modifications of approved CO_2 laser therapies of premalignant lesions resulted in higher recurrence rates than the conventional defocused laser technique. However, several studies indicate that other wavelengths such as Nd–YAG (λ=1,064 nm) or diode lasers (λ=810 nm) may be also of value in this field. In many other indications, the use of lasers is still experimental. Intraoperatively used photodynamic therapy or periimplant care of ailing implants with the CO_2 laser seems to be more of value than conventional methods. However, further studies are required to assess standard protocols. Over the past years, research identified some new indications for laser treatment in oral surgery and implant dentistry. Moreover, well-known laser applications were defined as state of the art. Nevertheless, further studies are required for laser treatment in oral surgery and implant dentistry.

Keywords Laser · Oral surgery · Implant dentistry

Introduction

This article is a comprehensive review of recent laser applications in oral surgery and implant dentistry, providing information for dentists and oral and maxillofacial surgeons. Therefore, the authors focus on new laser techniques in osteotomy, treatment of premalignant lesions, fluorescence spectroscopy and photodynamic therapy (PDT), periimplant care of ailing implants, and local hemostasis.

To understand the use of laser surgery, it is necessary to know the fundamental principles of laser light. Unlike other light sources, lasers emit coherent, monochromatic, and collimated electromagnetic radiation. These characteristics endow lasers with unique applications. The most common surgical lasers emit wavelengths in the infrared part of the spectrum: the neodymium:yttrium–aluminium–garnet laser (Nd–YAG, λ=1,064 nm), the erbium–yttrium–aluminum–garnet laser (Er–YAG, λ=2.94 μm), and the CO_2 laser (λ= 10.6 and 9.6 μm). Within the visible portion of the electromagnetic spectrum, argon lasers emit a light between 458 and 515 nm, and excimer lasers are located in the ultraviolet part of the spectrum (100 to 400 nm). Diode lasers emit wavelengths of λ=810 and 906 nm. In surgical

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indications, within the last years, the latter seem to be of increasing interest.

Whether a laser system is suitable for incisions, vaporization, or coagulation is determined by the wavelength, the energy fluence, the optical characteristics of the tissues, and how the laser is operated. In continuous mode, the laser provides a constant and stable delivery of energy. Pulsed laser systems, in contrast, provide bursts of energy. Lasers within the ultraviolet region (100 to 380 nm) are able to ionize tissues, a process known as photochemical desorption. Lasers of longer wavelengths, especially those within the infrared part of the spectrum (700 to 10,000 nm), cause significant tissue heating. Most of the surgical lasers are embedded in this group and comprised as thermal lasers. The light of these lasers is rapidly converted to thermal energy, causing denaturation of proteins, decomposition of tissue, microexplosion of cell water, and charring. However, recent studies showed that the CO_2 laser at 9.6 μm made an important step toward replacing conventional osteotomy techniques [1, 2].

New laser applications in oral surgery and implant dentistry

Laser osteotomy

For most patients, drills and hand pieces are the most inconvenient components in oral surgery. Therefore, laser osteotomy could be an elegant alternative [1–3]. Research was focused on most of the medically used laser systems. The major components of bone and dental hard tissues are inorganic structures such as water and hydroxyapatite as well as organic structures (collagen). Several authors described the critical temperature for bone and noted that temperature elevation between 44 and 47°C may lead to osteonecrosis [3]. The laser light emitted by the CO_2 and the Er–YAG laser are well absorbed by water. The wavelength of the Er–YAG laser, moreover, is well absorbed by water and hydroxyapatite. In addition to a high absorption coefficient for water and for hydroxyapatite with phosphate, carbonate, and hydroxyl groups, the energy emitted by the CO_2 laser at 9.6 μm is also highly absorbed by collagen. Therefore, this wavelength seems to play an increasingly important role in oral and maxillofacial surgery.

Eyrich [1] compared the super-pulsed CO_2 laser at 9.6 μm to the Er–YAG laser and the conventional drill with regard to their respective thermal effects on human bone. Therefore, temperature rise during ablation of human bone was measured. The results of the study suggested that a maximum rise of mean temperature to 1.88°C (well below the critical range of 7°C) demonstrated the safety and tissue-preserving capability of the super-pulsed 9.6-μm CO_2 laser. The laser caused an even lower temperature rise than conventional drilling when using this device for osteotomies on larger bone segments compared to small bone slices. Moreover, the laser showed acceptable efficacy with drilling times comparable to a conventional drill.

In another study [1], bony osteotomies were produced in six patients with 60-μs pulses of a pulsed 9.6-μm CO_2 laser and a scanning system. Histologic sections revealed no charring, but a very thin basophilic zone was seen next to the cut surface. Cutting trabecular structures resulted in a coagulation zone of 20–150 μm. The author concluded that clinical use of a 9.6-μm CO_2 laser as a cutting tool can be considered to preserve tissue with almost no adverse effects at the light microscopic level.

Lasers in premalignant lesions of the oral mucosa

According to the literature, malignant transformation of premalignancies such as oral leukoplakia and oral lichen planus occurs in up to 28% of these lesions [4]. Consequently, due to the high rates of malignant transformation and basically unchanged prognosis of head and neck cancer, early treatment of premalignant lesions is mandated. Even though there are some reports in the literature on laser-assisted tumor treatment, surgery is mostly performed conventionally. As an alternative to the scalpel, the CO_2 laser (λ=10.6 μm, continuous wave, defocused) is an established device which has been in use for more than 20 years. It has been demonstrated histologically that thermal laser energy carbonizes superficial parts of epithelium. Consequently, reepithelization is delayed for more than 2 weeks. This technique has been proven very effective being associated with recurrence rates of less than 20% [5].

However, a delay in healing caused by the thermal laser energy is an encumbrance for the patient. Therefore, new methods of applying laser energy, such as scanners or the use of very short laser pulses (the so-called super pulses, sp), could be of value. Scanners allow the focused CO_2 laser beam to sweep quickly over an area, thereby reducing the dwell time on each individual point to less than 1 ms which is shorter than the thermal relaxation of soft tissue (3.6 ms) [6]. Through the use of the sp-mode as well as the scanners, thermal laser effects such as delays in healing can be reduced but, on the other hand, a lesser degree of destruction of dysplastic cells could lead to an increased recurrence rate.

Accordingly, the aim of a recent study was to evaluate the recurrence rates resulting from different methods of CO_2 laser surgery in a prospective clinical study. Therefore, a total of 56 patients with a total of 68 premalignant lesions of the oral mucosa were treated with three different modes of CO_2 laser surgery [5]. In the group with defocused resection of oral leukoplakias, a recurrence rate of 23.1%

was seen, which is very similar to that found in the literature [4, 7]. In contrast, neither the application of scanner plus cw-irradiation nor the scanner plus sp-mode yielded results superior to those of the classic defocused technique. These results were explained by the pulsed mode of laser beam delivery and, furthermore, the geometry of the laser beam on the scanned area.

Oral lichen lesions were associated with very high recurrence rates. According to the literature, oral lichen is an autoimmune disease which is not amenable to healing by means of resection. Consequently, only erosive lesions should be treated to achieve pain relief for the patient.

Tissue effects resulting from different scanning systems were also assessed in an experimental study [8]. Therefore, healing of skin wounds after CO_2 laser resection was evaluated with the use of two different scanners (Swiftlase® and Silktouch®). Histologically and clinically, both scanners yielded better results with regard to progress of wound healing than those seen with the use of a defocused laser beam. Nevertheless, these differences could no longer be detected at 2 weeks after surgery. Due to the digitally generated mode of the laser beam on the irradiated area, smoother skin surfaces were yielded with the Silktouch® scanner.

In recent studies, very low recurrence rates were observed with the Nd–YAG laser (λ=1064 nm) [9] and a diode laser (λ=810 nm) [10]. At these wavelengths, laser energy is not absorbed to any significant extent in water. As a result, deleterious effects on sensitive structures such as the mental nerve might occur. Nevertheless, the use of these wavelengths for resection of premalignant lesions should be evaluated in subsequent studies.

Lasers in fluorescence spectroscopy and PDT

Laser-induced fluorescence (LIF) spectroscopy is a noninvasive technique that has been used in various fields to differentiate tissues and, therefore, might be an important tool for cancer diagnostics. In a recent pilot study, the ability of LIF spectroscopy to detect dysplasia or cancerous tissue was validated [11]. Therefore, a 337.1-nm nitrogen laser with a 600-μm fiber optic was used to induce fluorescence in human normal and pathological tissues. Fluorescence spectra were obtained by means of a spectrograph and analyzed by a computer program. The results of this study indicated that differentiation of benign and malignant tissues was possible with a sensitivity above 80%. The authors concluded that this method might be applicable for discrimination of benign and malignant tissues. It was stated that LIF spectroscopy may provide the clinician with a reliable technique for detecting malignancies. Nevertheless, the authors recommended further studies to verify the in vivo applicability of the method.

It has been shown in the past that PDT can optimize conventional surgery in squamous cell carcinoma [12–14]. In a recent animal study, PDT has also been performed intraoperatively next to vital structures like the carotid artery using a new photosensitizer meta-tetrahydroxyphenylchlorin (m-THPC) [14, 15]. As a result of the irradiation, complete necroses of muscles and connective tissue were found. Nerve tissues demonstrated demyelination (above 75%), however, without clinical symptoms.

Intraoperative PDT using m-THPC has also been performed in 22 patients with malignancies of the brain [16]. The authors concluded that m-THPC-mediated, intraoperative fluorescence-guided resection followed by PDT is a highly promising concept in improving the radicality of tumor resection combined with a therapeutic approach.

Nevertheless, more studies are necessary before these methods can be recommended as standard therapies in the treatment of oral carcinoma.

Periimplant care of ailing implants

A new indication of laser treatment might be the sterilization of exposed implant surfaces to rehabilitate ailing implants. However, apparently not all laser systems available in dentistry are of value in this regard. Park et al. [17] reported that the potential exists for Nd–YAG laser irradiation (λ=1064 nm) to melt the surface and even to remove the surface layer from plasma-coated titanium implants. From this study, it was concluded that the use

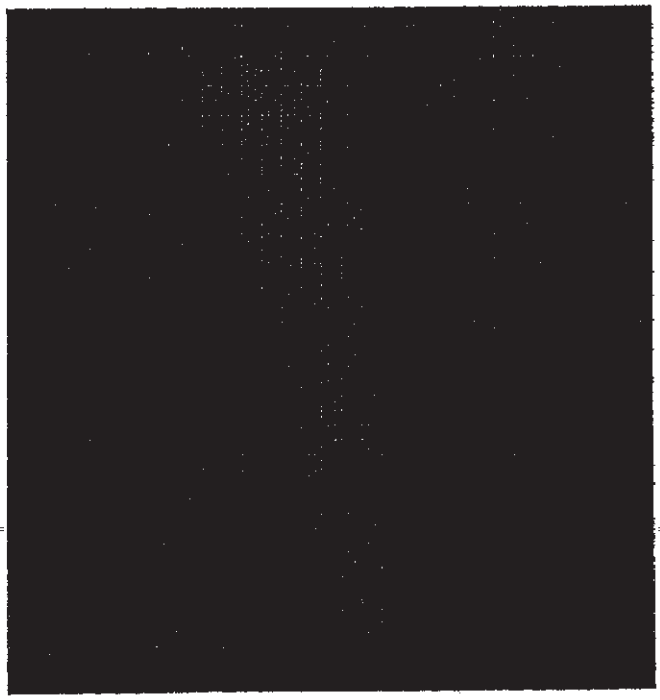

Fig. 1 Radiograph indicating chronically progressive periimplant bone resorption

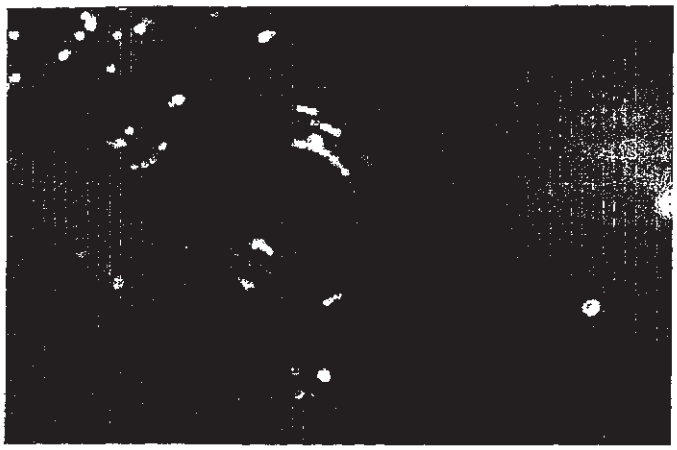

Fig. 2 Surgical intervention: full thickness flaps and granulation tissue removal

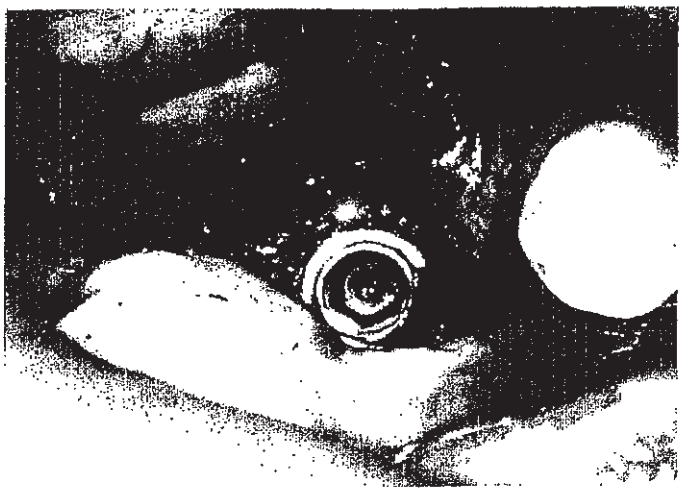

Fig. 4 Reentry 4 months after therapy. Complete closure of the defect

of Nd–YAG lasers in implant-uncovering procedures or periimplant gingival surgery should be considered inherently unsafe for such procedures.

Better results were seen with the use of a CO_2 laser (λ= 10.6 μm). The purpose of a study in a total of 16 patients with 41 ailing implants was to assess the reliability of the CO_2 laser-assisted implant decontamination vs a conventional decontamination procedure [18]. The results of the clinical study showed, 4 months after therapy, that implants treated with laser decontamination and soft-tissue resection exhibited statistically significant better clinical parameters than conventionally decontaminated implants followed by soft-tissue resection. From these results, it was concluded that treatment of periimplantitis can be optimized using a CO_2 laser-assisted decontamination (Figs. 1, 2, 3, 4, and 5).

There are several positive reports in the literature in which laser decontamination has been recommended including the use of diode lasers (λ=810 and 906 nm) [19–21] and Er–YAG laser (λ=2.94 μm) [22]. Application of a diode laser (λ=810 nm) resulted in recurrence rates of less than 7% [19]. In further studies, PDT with toluidine blue plus diode laser light (λ=906 nm) was used [20, 23]. Haas and coworkers [20] reported on a mean bony reapposition of 2 mm (±1.90 mm) after a 9.5-month observation period. However, reosseointegrations were demonstrated for the first time for the CO_2 laser [6]. Most

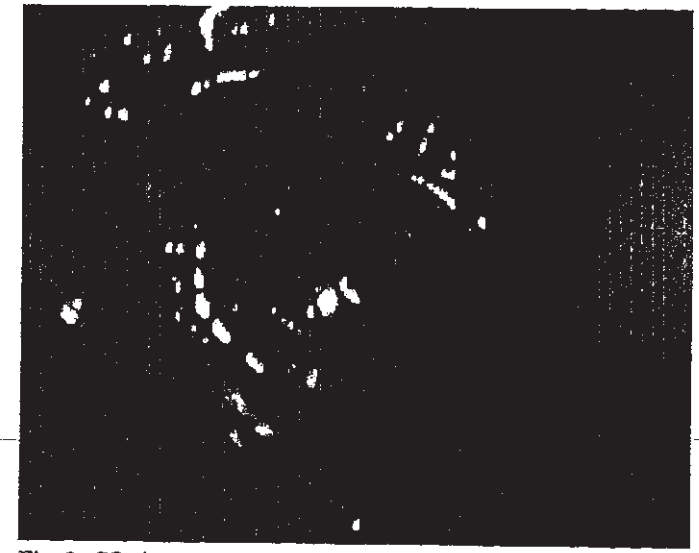

Fig. 3 CO_2 laser-assisted implant decontamination and augmentation with beta-TCP

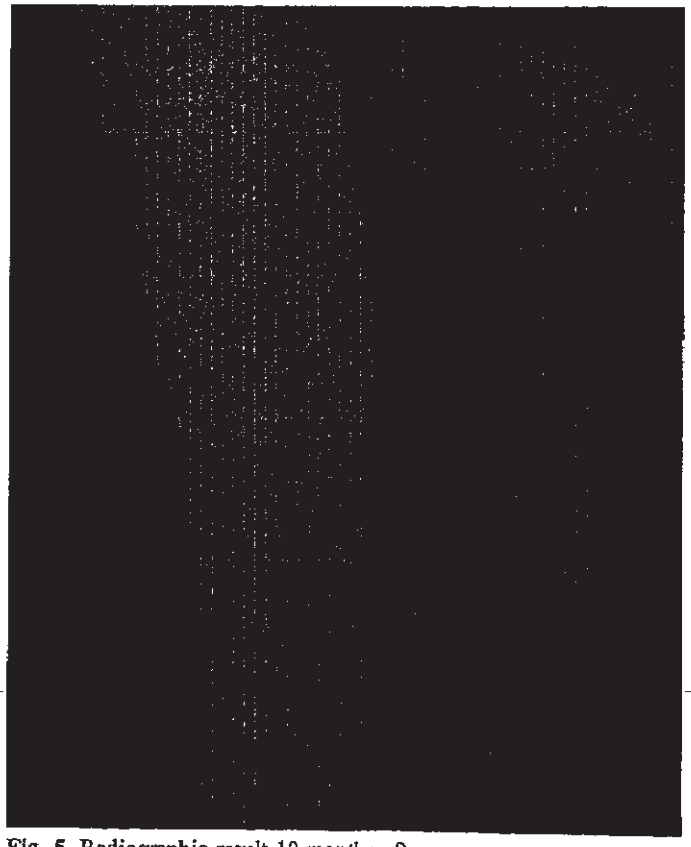

Fig. 5 Radiographic result 10 months after surgery

recent results from a study performed in beagle dogs have indicated that reosseointegration also occurred after irradiation with an Er–YAG laser [24]. Nevertheless, further studies are required in this field.

Bare fiber technique in local hemostasis

In modern societies, there is an increasing number of older patients, especially those treated with anticoagulation because of cardiologic indications. Over the past years, laser hemostasis has been established as an alternative to conventional techniques. Due to a penetration depth of more than 4 mm in soft tissue, cw Nd–YAG laser light (λ=1064 nm) applied with a hand piece has been very effective in this field [25].

However, if bleeding occurs massively from the apical region of the socket, the use of the bare fiber can be of interest. Therefore, in a clinical study in 44 patients, the bare fiber technique was studied in this indication [4]. Moreover, to reduce the thermal effects, a pulsed laser was used. It was concluded that intraalveolar application of pulsed Nd–YAG laser energy can be considered safe. It was demonstrated that optical characteristics of blood result in scattering and dispersion of laser light, thereby reducing the adverse effects on bony tissue.

Conclusion

Over the past years, research identified some new indications and techniques for laser treatment in oral surgery and implant dentistry. Moreover, well-known laser applications were defined state of the art. Nevertheless, further studies are required for laser treatment in oral surgery and implant dentistry.

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Decontamination of rough titanium surfaces with diode lasers: microbiological findings on *in vivo* grown biofilms

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Key words: decontamination, diode laser, oral biofilm, titanium surface

Abstract

Objectives: The bactericidal efficacy of diode lasers has already been demonstrated *in vitro*. We investigated the reduction of aerobe bacteria – colonizing rough titanium samples in biofilms intraorally grown – by diode lasers of different wave lengths.

Material and methods: Twenty-two volunteers participated in the trial. They were fitted for 10 days with custom-made intraoral plastic splints carrying titanium sleeves. A part of the sleeves was then irradiated with diode lasers in different modes. The other part remained non-irradiated and served as control. Directly after irradiation, the sleeves were swabbed and the gained bacteria were first examined microscopically and then were cultured under aerobic conditions.

Results: The bacteria in the controls and in the treated samples were quantified. A comparison with the controls revealed a marked overall reduction of bacterial colonization in all irradiated sleeves. Continuous irradiation for 20 s reduced bacteria counts by 99.67% at 810 nm and 99.58% at 980 nm. Repeating the 20 s exposure five times reduced counts by 99.98% at 810 nm and by 99.39% at 980 nm. A 98.86% reduction was seen after irradiation in pulsed mode. A further analysis in respect of different isolated bacteria revealed that the streptococci group was reduced by 99.29–99.99%, while the staphylococci group was reduced to a lesser extent in the range 94.67–99.99%.

Conclusion: The results are of clinical relevance. In comparison with the mean bacterial counts of the untreated samples, all irradiation programs studied in this investigation reduced mean bacterial colonization in a biofilm on intraoral rough titanium surfaces by more than 98%. The actual extent of reduction was dependent on the bacteria species as well as on the irradiation mode.

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Decontamination of the implant surface is one facet in the therapy of peri-implantitis, one of its goals is to reduce the number of colonizing pathogens as much as possible. The causal relationship between a persisting biofilm on the implant surface and the occurrence of peri-implant inflammation has been established clinically (Mombelli & Lang 1998; Hultin 2002; Shibli et al. 2003). Different microorganisms have been detected at peri-implant sites (Lee et al. 1997; Hultin 2002; Leonhard et al. 2003; Shibli et al. 2003). This bacterial contamination is connected to peri-implant infections and sometimes causes even implant failure (Rams & Link 1983; Becker et al. 1990; George et al. 1994; Piattelli et al. 1998; Leonhardt et al. 1999, 2003). The environmental conditions of the biofilm lead to increased resistance to antimicrobial treatment (Anwar et al. 1992; Larsen & Fiehn 1996;

Souli & Giamarellou 1998; Sbordone & Bartolaia 2003).

The antimicrobial activity of laser light, which depends on its photothermic effects, has been described by a number of authors *in vitro* (Deppe et al. 2001; Sennhenn-Kirchner et al. 2002; Kreisler et al. 2003; Soukos et al. 2003; Romanos et al. 2004) and *in vivo* (Moritz et al. 1997; Romanos & Nentwig 1999; Bach et al. 2000; Haas et al. 2000; Deppe et al. 2002). The antimicrobial efficacy of the diode lasers has been previously demonstrated *in vitro* (Sennhenn-Kirchner et al. 2002; Kreisler et al. 2003). The evaluation of the efficacy of diode laser light on biofilms induced *in vivo* is missing up to now. The present study investigated the decontaminating effect of five different irradiation programs of two different diode lasers (one emitting light at a wavelength of 810 nm and one at 980 nm) on intraorally grown biofilms on rough titanium surfaces. This study using a model of 'old' biofilm (Anwar et al. 1992) grown under *in vivo* conditions adds new data on proofs of laser efficacy published until now with *in vitro* models. The results prove the efficacy of both wavelengths with regard to the reduction of biofilm producing aerobe bacteria.

Material and methods

Laser and laser programs

The antimicrobial effect of five different diode laser irradiation programs with two different diode lasers: (1) 810 nm wavelength Ora Laser 01 I.S.T., Oralia, Konstanz, and (2) 980 nm wavelength Schütz WDL 2.5, Schütz Dental Group were studied.

(1) 810 nm wavelength, continuous wave (cw) mode with 1 W, 600 µm wave guide fiber for 20 s.
(2) 810 nm wavelength, cw mode with 1 W, 600 µm fiber for 20 s repeated five times with a 30 s pause after 20 s irradiation time.
(3) 980 nm wavelength, cw mode with 1 W, 500 µm fiber for 20 s.
(4) 980 nm wavelength, cw mode with 1 W, 500 µm fiber for 20 s repeated five times with a 30 s pause after each 20 s irradiation time.
(5) 980 nm wavelength, pulsed mode (1.5 W, 20 Hz, 3 ms), 500 µm fiber for 20 s repeated five times with a 30 s pause after each 20 s irradiation time.

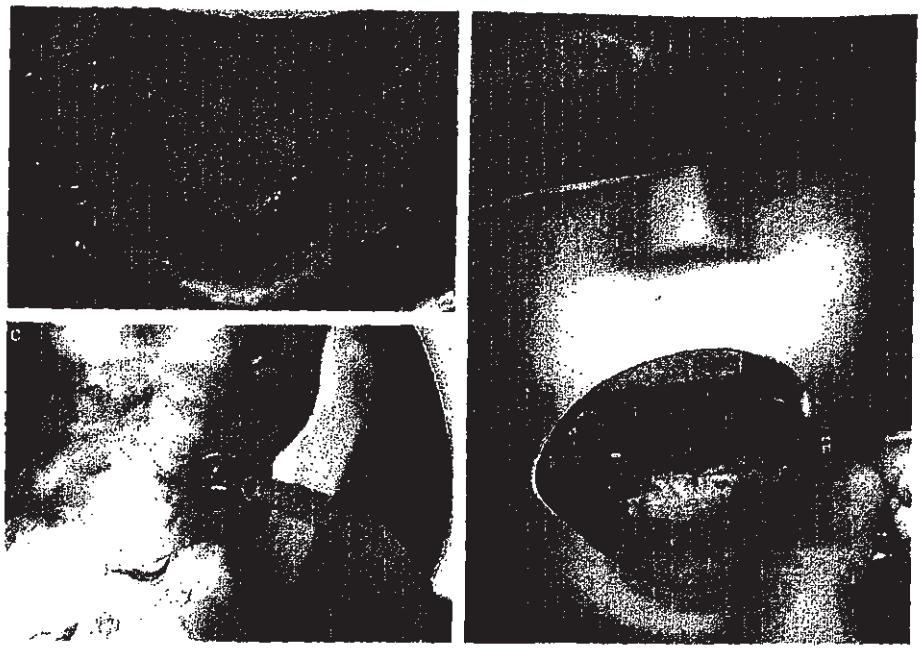

Fig. 1. (a) Titanium sleeves attached to custom-made plastic splints by light-curing resin. (b) Splint mounted on custom-made plaster model in a phantom head. (c) Application of the laser beam to the titanium surface.

Study objects and study design

Twenty-two volunteers participated in the trial. The study objects were titanium sleeves (Steco, Hamburg, Germany), normally used as drill guide for dental implantology (outside diameter of 3 mm, inside diameter of 2.35, 5 mm long) which were sand-blasted before use (Alcastral 150 µm, OmniDent, Rodgau Niederöden, Germany). In order to ensure a secure intraoral position, the sleeves were attached in vertical position to the buccal sides of custom-made mandibular plastic splints (Erkodur resin foil, 120 mm, Erkodent, Pfalzgrafenweiler, Germany) with light-curing resin (Triad Gel Clear Colorless, Dentsply, Konstanz, Germany). Figure 1a shows the top view on the splint *in situ*.

The splints were fitted to the patients mandible and they remained in place for 10 consecutive days and nights. They were removed from the oral cavity only for tooth brushing, interdental flossing, and for the intake of food and liquids, and then too for a longest time of 90 min at a time. During this time, they were stored in sterile plastic bags. The use of any kind of mouth rinse was prohibited during the entire period. After the 10-day period, the splints were removed and mounted on custom-made plaster models in a phantom head (Fig. 1b). Each voluntary participant carried at least three sleeves in his mouth.

One titanium sleeve from each splint was left untreated and served as control. Two sleeves from the same person were treated with two of the diode laser programs described above. The allocation of the different programs were random. Every program (1–5) was applied in eight test persons, so that eight different test parameters were gained for every program. All treatments were performed under identical conditions by the same investigator. The laser beam was applied to the inner titanium surface of the sleeves with an up-and-down motion in slight contact mode (Fig. 1c).

Samples and microbiology

Immediately after irradiation, swabs were obtained from each titanium sleeve with sterile tweezers and by scrubbing with sterilized interdental brushes (Cupaprox CPS 12 regular, Curaden, Kriens, Switzerland), exactly fitting in the sleeves in diameter, and with exactly 10 strokes per sleeve (Fig. 2). The swabs were placed in sterile Eppendorf tubes containing 1000 µl physiological saline according to Kite et al. (1997). The dissolved material was mixed

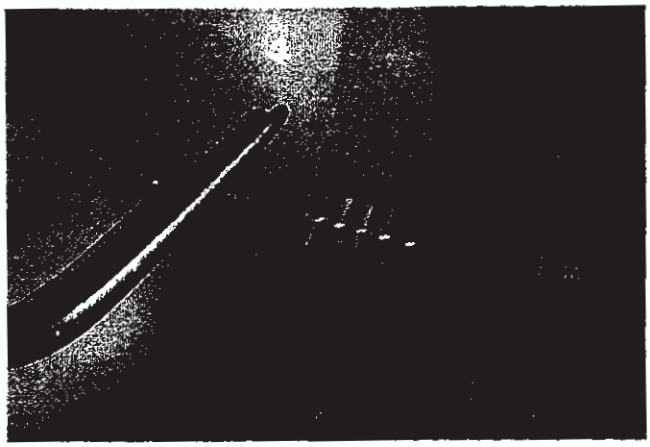

Fig. 2. Swabs were obtained from each titanium sleeve.

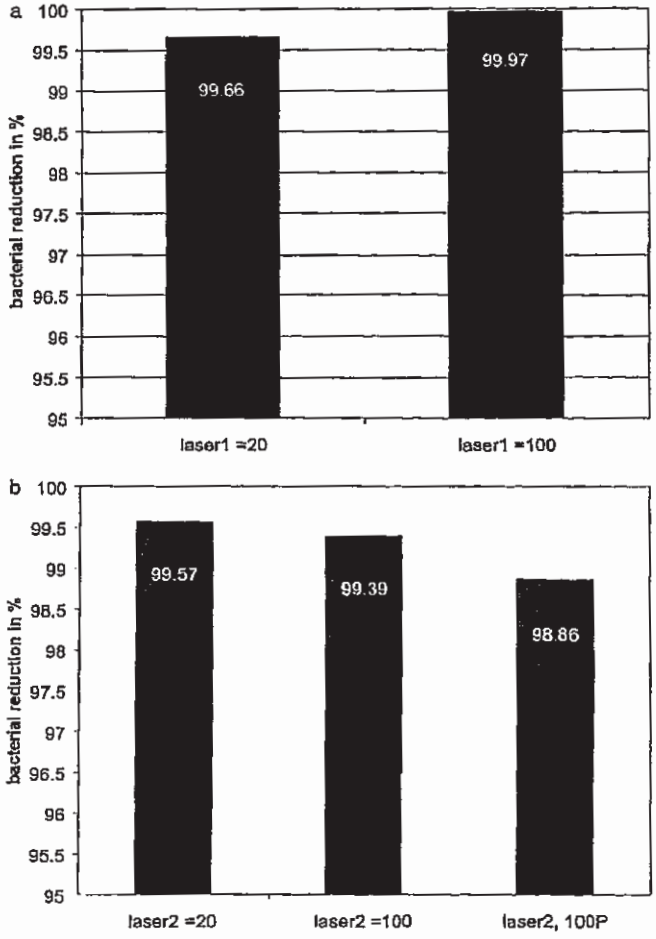

Fig. 3. (a) Effect of irradiation regimen by diode laser at 810 nm wavelength. Comparison of two application modes: continuous wave for 20 s (=20) and 100 s (=100). The figure shows the percentage of the mean bacterial reduction of each regimen compared with untreated controls that were set to 0%. To demonstrate the small differences between these two regimens, the *y*-axis starts at 95%. (b) Effect of the irradiation regimen performed with the diode laser at 980 nm wavelength. Comparison of three different application modes: 20 s (=20) and 100 s (=100) and pulsed mode (=100p). As in Fig. 3a the percentage of the mean bacterial reduction of each regimen is demonstrated and the *y*-axis starts at 95% to reveal the small differences between these regimens.

on a vortexer (VF 2, Anke and Klunke, Staufen i. Br., Germany) for 1 min. For the determination of the bacterial concentration of the different samples, the dissolved material was serial diluted in physiological saline (10^{-1}– 10^{-5}) following the method of Süßmuth et al. (1999). One hundred microliter aliquots of each dilution were then plated on blood agar plates (Columbia agar, Bio Mericux, No. 43049, Marcy L'Etoile, France) and incubated under aerobe conditions at 35 ± 1°C for 24 h (Reach-in incubator, Forma Scientific, Marietta, OH, USA). Colony-forming units (CFU) were counted by a colony counter (Bio, Kobe, Japan).

The CFU were analyzed for morphological differences on the agar plates and were first classified by Gram staining (Süßmuth et al. 1999). The bacteria were further differentiated by their metabolic properties with a commercially available identification system (BD BBL Crystal GP, No. 245140, Becton Dickinson, Heidelberg, Germany) after incubation for 24 h at 35 ± 1°C under aerobic conditions.

Statistics

The mean decontamination rates were calculated for each program separately and statistical analysis was performed as follows: In order to compare the paired observations of the modes of laser 1 (control, 20 and 100 s) Friedman's test and for comparisons in pairs the sign test were applied using the closure principle to adjust for multiple testing. For the independent observations of laser 2, a non-parametric ANOVA with two fixed factors (laser mode and group) was used. Again comparisons in pairs were adjusted for multiple testing using the closure principle.

Results

All laser irradiation regimens used in this investigation had marked antimicrobial effects on the detected bacteria gained from an intraorally grown biofilm when compared to the controls (Fig. 3a, b). The reduction rates were statistically significant.

- control/laser 1: $P<0.0001$,
- control/laser 2 cw: $P<0.0001$,
- control/laser 2 – 100p: $P<0.0001$.

Laser 1 (810 nm wavelength) induced an average CFU reduction of 99.66% with an

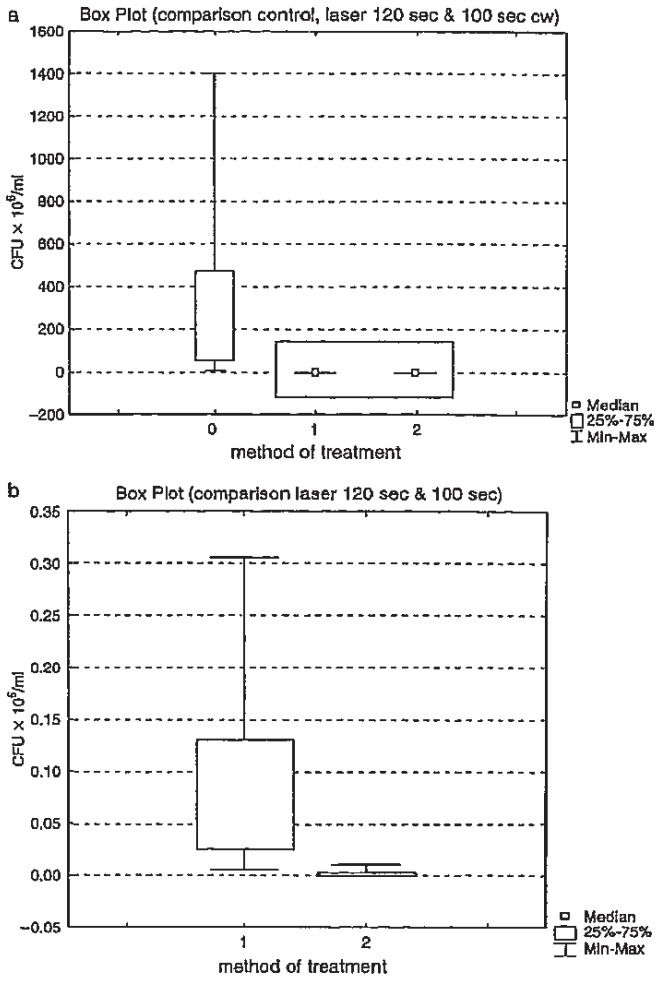

Fig. 4. Summary of the results gained by diode laser at 810 nm wavelength in detail. (a) Box plot of the results gained from at least eight different samples in each group. The bacterial counts of untreated controls (0) (median: 31.67 × 10^6 CFU/ml; interquartile range: 37.16; standard deviation: 22.77) is demonstrated, as well as the results obtained by the different programs 1 and 2 of this laser (compare (b)). (b) Box plot of the exact results gained with laser program 1 and program 2. To show the differences between both programs a different scale of the *y*-axis has been chosen. With program 1 a median of 0.034 × 10^6 CFU/ml (interquartile range: 0.29; standard deviation: 0.113) was obtained, while program 2 revealed a median of 0.00035 × 10^6 CFU/ml (interquartile range: 0.01; standard deviation: 0.0039). However, the differences between the programs, and between each program and the respective control were significant (Friedman test; sign test).

average reduction rate of 2.96 $\log_{10}$ steps when applied for 20 s at 1 W in cw mode.

Repeating the treatment five times increased the average CFU reduction rate to 99.98% (3.34 $\log_{10}$ steps). The increase in CFU reduction by the repetition of irradiation was statistically significant, $P = 0.0156$/Friedman's test, followed by sign test (Fig. 4a, b).

- laser 1 −20/−100: $P = 0.0156$.

Laser 2 (980 nm wavelength applied for 20 s at 1 W) induced an average CFU reduction of 99.57% with a rate of 2.89 $\log_{10}$ steps. Increasing the application time to five times 20 s showed an average CFU reduction of 99.39%, while the pulsed mode at 1.5 W and five times 20 s irradiation induced a reduction of 98.86%. The differences between these programs were not statistically significant:

- laser 2 −20/−100 cw: $P = 0.60$,
- laser 2 −100 cw/−100p: $P = 0.61$.

Various species of staphylococci and streptococci were detected in the biofilms. In all participants, streptococci occurred, which could not be identified by the used test kit. In combination with these streptococci, microorganisms could be detected. Sometimes they occurred alone and sometimes together with other bacteria as follows:

- *Staphylococcus aureus* (five cases) in combination with *S. lentus*, *Streptococcus pneumoniae* and *Str. vestibularis*,
- *S. crista* (three cases), once combined with *Str. pneumonia*,
- *Str. pneumoniae* (five cases) in combination with *Lactococcus lactis*, *S. aureus*, *S. crista* and *Str. vestibularis*,
- *Str. sanguis* (in two cases) and with *S. auricularis* and *S. hämolyticus*,
- *Aerococcus urinae* (in four cases) with *Micrococcus luteus*, *L. lactis* and *Str. Pneumonia* and
- *L. lactis* (in five cases).

Mean bacterial reduction rates were clinically relevant and could be measured between 94.67% and 100%. It depended on applied laser irradiation regimen and on bacterial species or even subtype; staphylococci showed minor decrease rates than other species. Comparison of medians and confidence intervals showed no relevant differences (Table 1).

Discussion

Following the demonstration of the antimicrobial efficacy of diode laser light *in vitro* by Sennhenn-Kirchner et al. (2002) and Kreisler et al. (2003), it is an open question whether it might be effective against bacteria protected by *in vivo* grown biofilms. Biofilms have been characterized by *in vitro* (Xu et al. 2000; Donlan & Costerton 2002; Pratten et al. 2003) and *in vivo* research (Marsh 1995; Bradshaw et al. 1997; Socransky et al. 1998, 2004; Soukos et al. 2003). Referring to these examinations and following the arguments of Costerton & Lewandowsky (1995) and Costerton et al. (1999), it can be assumed that pathogens associated with peri-implant infections are protected by biofilms (Bradshaw et al. 1997; Hultin 2002). Biofilm-producing bacteria are able to colonize all intraoral surfaces, particularly rough structures, such as the surface of implants (Krekeler et al. 1990; Marsh 1995; Bollen et al. 1996; Lee et al. 1997; Mombelli & Lang 1998; Groessner-Schreiber et al. 2004; Kuula et al. 2004). Biofilms protect

Table 1. Mean reduction (MR) of different species of bacteria by different laser irradiation regimens in percent related to the mean basic bacterial counts of the untreated samples, medians and confidence intervals (CI)

	Laser 1=20	Laser 1=100	Laser 2=20	Laser 2=100	Laser 2; 100 P
Staphylococci (11 P) MR	99.44	99.99	99.99	97.62	94.67
Median	99.44	100	99.99	99.99	99.13
CI	92.4–106.5	99.99–100	99.96–100	93.6–101.6	84.1–105.25
Streptococci (22 P) MR	99.29	99.99	99.8	99.94	99.94
Median	99.94	100	99.87	100	100
CI	98.2–100.37	99.98–100	99.65–99.96	99.82–100	99.87–100
Aerococcus urinae (4 P) MR	100	/	99.92	100	99.99
Median	100	/	99.94	100	99.99
CI			99.72–100.13		99.98–100.02
Lactococcus lactis (5 P) MR	99.75	99.95	100	100	99.95
Median	99.79	99.93	100	100	99.95
CI	99.25–100.25	99.83–100.06			99.57–100.33
All bacteria (22 P) MR	99.67	99.98	99.58	99.39	98.78
Median	99.93	100	99.95	100	99.98
CI	98.78	99.97–99.99	99.6–99.98	98.1–100.35	96.67–100.72

P, participants.

the colonizing microorganisms against a wide variety of exogenous influences (Anwar et al. 1992; Souli & Giamarellou 1998; Cochran et al. 2000; Sbordone & Bartolaia 2003; Soukos et al. 2003). Donlan & Costerton (2002) reviewed literature on survival mechanisms of clinically relevant microorganisms in biofilms. The microorganisms that grew in biofilms express a distinct phenotype that made them resistant to antibacterial agents and host response. Therefore the therapeutic success of infections caused by bacterial biofilm colonization of surfaces is more difficult to achieve. It has been shown that for the eradication of bacteria in biofilms, antimicrobial agents have to be overdosed up to 10 times (Wilson 1996; Socransky et al. 2002). In these cases the use/risk factor for the patient may easily shift to damage. So the efficacy of laser irradiation under different therapeutic aspects has to be investigated.

Following the arguments of Hejdenrijk et al. (2002) basing on studies of Quirinen & Listgarten (1990), Leonhardt et al. (1999) and Rosenberg et al. (1991), the simple presence of pathogens at peri-implant sites will not cause peri-implant infections consecutively as long as the number of these periodontal pathogens is kept at a low level and other potential (co)-factors are within normal limits. This emphasizes the necessity of reducing bacteria at peri-implant sites.

The present study investigated the decontamination efficacy of various diode laser irradiation programs on aerobic bacteria. The composition of subgingival biofilms has been described frequently (Socransky et al. 1998; Rutar et al. 2001; Leonhardt et al. 2003). A primary colonization has been demonstrated with cocci (Shibli et al. 2003; Li et al. 2004). Cocci seem to pave the way for colonization with anaerobic organisms (Rams et al. 1990; Wu-Yuan et al. 1995) and they are used for biofilm related studies. Anaerobes are very sensitive to oxygen. Therefore it has to be assumed that the yield of anaerobes gained by the microbrush technique might be too low leading to a false-positive effect of the laser therapy. Many studies on this topic focus on anaerobes considered to be involved in the etiology of peri-implant infections (Bollen et al. 1996; Lee et al. 1997; Rutar et al. 2001; Hultin 2002; Socransky et al. 2004), and rely on molecular biological analysis. However, some studies have demonstrated differing floras associated with periodontitis and peri-implantitis (Rams et al. 1990; Rutar et al. 2001; Leonhardt et al. 2003). Leonhardt et al. (1999, 2003) found approximately equal numbers of anaerobic microorganisms on the one hand, and aerobic cocci and yeasts on the other in infected peri-implant sites by cultivation and plating.

In this study we focused on cocci to evaluate the decontamination effects of laser light. These cocci had grown in biofilms on rough titanium surfaces which had been positioned intraoral in various voluntary persons. Therefore, the obtained biofilms showed differences in their composition of bacteria.

The efficacy of laser light of various wavelengths to decontaminate surfaces has been demonstrated repeatedly *in vitro* (Coffelt et al. 1997; Haas et al. 1997; Kreisler et al. 2002a, 2002b, 2003; Sennhenn-Kirchner et al. 2002).

Its clinical use in the treatment of peri-implantitis has been described (Bach et al. 2000; Haas et al. 2000; Shibli et al. 2003), but there are hardly any studies on the direct effects of laser light on biofilms as the literature reviews show (Roos-Jansacker et al. 2003; Esposito et al. 2004). Rovaldi et al. (2000), for example, found a 6 log bacterial decrease by photosensitation and following 662 nm laser irradiation *in vitro*. However, the same treatment mode applied on plaque bacterial biofilm samples of periodontal affected persons leads just to 75–92% reduction which means a ≤ 2 log decrease (Soukos et al. 2003).

As was shown previously *in vitro* (Haas et al. 1997; Goharkhay et al. 1999; Sennhenn-Kircher et al. 2002; Kreisler et al. 2003), applying diode laser light, either 810 or 960 nm wavelength in a continuous mode was highly effective. The light of the diode laser with 1 W of power has only little thermal penetration, which obviates possible injury to oral tissue or damage to the titanium (Romanos et al. 2000; Kreisler et al. 2002b). One would, therefore, expect no risks from its clinical application (Goharkhay et al. 1999; Kreisler et al. 2002b; Romanos et al. 2004).

The study design imitated the conditions encountered clinically in the treatment of peri-implantitis. However, there are differences between the surface structure of the study objects and the implants requiring treatment in clinical practice. In general,

cocci predominate in biofilm formation, especially at the beginning as Leonhardt and others were able to demonstrate.

A threaded implant has a far larger surface area than that of the roughened titanium sleeves, and not all areas are accessible in the same intensity by laser irradiation due to the threads. On the other hand, irradiation of the study objects was impaired not only by poor visibility but also by the small inside diameter, and that it was not possible to apply the light to the surface at the optimal angle of 90°.

The results of this study prove diode laser light highly effective, as had already been demonstrated *in vitro*. However, the successful eradication of biofilms is much more difficult (Anwar et al. 1992; Costerton & Lewandowsky 1995), but following the results of this study, pathogens grown intraorally in biofilms are highly injured by the application of laser light.

Conclusions

The results of this study prove the investigated treatment modes effective for the reduction of aerobic bacteria on rough titanium surfaces although protected by accumulation in intraorally grown biofilms. Compared with the mean bacterial counts of untreated controls (0% reduction), laser irradiation treatment reduced the mean bacterial counts in the range 98.86%–99.98%. Diode laser irradiation has been proven an instrument for significant bacterial reduction even when microorganisms are consolidated in a ten days old biofilm. It remains to be determined whether this treatment is just as effective in the clinical treatment of peri-implant defects in inaccessible areas and in decontaminating the implant threads.

要旨
目的：ダイオード・レーザーの殺菌効果はインビトロの研究において証明されている。我々は、異なる波長のダイオード・レーザーを用いて、口腔内で増殖したバイオフィルム中の粗いチタン試料上でコロニー化した好気性細菌の減少を調べた。
材料と方法：ボランティア２２名が本研究に参加した。被験者は１０日間チタン・スリーブを取り付けた特製のプラスチック・スプリントを口腔内に装着した。スリーブの一部に異なる様式でダイオード・レーザーを照射した。他の部分は照射を行わず対照とした。照射直後にスリーブを綿棒でこすって細菌を採取し、まず顕微鏡で検査し、次に有酸素下で培養した。
結果：対照試料と処置を行った試料の細菌数を数えた。対照と比較して、照射したスリーブでは全て細菌のコロニー化が顕著に減少していた。２０秒の連続照射では、細菌数が８１０nm で９９．６７%、９８０nm で９９．５８%減少した。２０秒照射を５回反復すると、細菌数は８１０nm で９９．９８%、980nm で９９．３９%減少した。パルス波照射後に９８．８６%の減少が認められた。異なる分離菌をさらに分析することによって、連鎖球菌群は９９．２９～９９．９９%減少したが、ブドウ球菌群は９４．６７～９９．９９%と減少の度合いが少ないことが分かった。
結論：同結果の臨床的な意義として、本研究の全ての照射プログラムは、未処置試料の平均細菌数に比べて、口腔内の粗いチタン表面上のバイオフィルム中の平均細菌コロニーを９８%以上減少させた。実際の減少の度合いは細菌種及び照射の様式に依存していた。

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