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

**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 810nm 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) 810nm and Er:YAG laser 2,940nm were employed for the LBC technique. Fibroblastic proliferation covering the entire wound was observed two days after treatment. 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.
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. Tooth-brushing near the site should 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.

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Fig. 9 Fig. 10 Fig. 11

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

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 eighth 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

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.
later been complemented by CO2 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. It could be demonstrated that especially the gram-negative, anaerobe microbiological spectrum was properly damaged by laser light (Bach und Krekel) in 1995; 2000). In compliance with reasonable performance 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 artificial 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 microorganisms 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.

**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.

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

<table>
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<tr>
<th>Age</th>
<th>Number of patients</th>
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<tr>
<td>20–30 years</td>
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<tr>
<td>30–40 years</td>
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<tr>
<td>40–50 years</td>
<td>3</td>
</tr>
<tr>
<td>50–60 years</td>
<td>2</td>
</tr>
<tr>
<td>60–70 years</td>
<td>1</td>
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<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of patients</th>
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<tr>
<td>Female</td>
<td>5</td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
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**Fig. 2.** Evaluation according to the sex of the examined and treated patients.
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, $t_{\text{max}} = 20$ 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 orthopantomogram 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 progression, 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 manufacturing company for microbiological diagnosis. The company is in charge of microbiological diagnosis and evaluation of the so-called probe 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, pulp, periimplantary and periodontal tissue structures will not suffer any thermic damages (Bach and Krekel (1995)).

_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-
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.

Date: preoperative 4 weeks p.o. 1 year p.o. 5 years p.o. 10 years p.o.

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<tr>
<td>Fusobacteria</td>
<td>2n/3m/1h</td>
<td>2n</td>
<td>2n</td>
<td>2n/1m</td>
<td>2n/1m</td>
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<tr>
<td>Prevotella intermedia</td>
<td>4n/2m</td>
<td>1n/1m</td>
<td>2n/1m</td>
<td>2n/2m</td>
<td>2n/2m</td>
</tr>
<tr>
<td>Porphyromonas ging.</td>
<td>2n/4m/2h</td>
<td>1n/1m</td>
<td>2n/1m</td>
<td>2n/2m/1h</td>
<td>2n/2m</td>
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(Legend: k.N. = no findings; n = low; m = middle; h = high)
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Decontamination efficacy of erbium:yttrium–aluminium–garnet and diode laser light on oral Candida albicans isolates of a 5-day in vitro biofilm model

Sabine Sennhenn-Kirchner · Peter Schwarz · Henning Schliephake · Frank Konietschke · Edgar Brunner · Margarete Borg-von Zepelin

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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

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 immuno-compromised 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 × 10⁷ 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/cm².
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/cm² and 15.2 J/cm².

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 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
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. 3 c 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.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Treatment</th>
<th>Mean value, 492 nm</th>
<th>SE</th>
<th>Min</th>
<th>Max</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>Control</td>
<td>0.25</td>
<td>0.04</td>
<td>0.20</td>
<td>0.31</td>
<td>Control/diode, ( P&lt;0.0001 )</td>
</tr>
<tr>
<td>Glass</td>
<td>Diode</td>
<td>0.03</td>
<td>0.03</td>
<td>0.010</td>
<td>0.06</td>
<td>Control/Er:YAG, ( P&lt;0.0001 )</td>
</tr>
<tr>
<td>Glass</td>
<td>Er:YAG</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.02</td>
<td>Diode/Er:YAG, ( P&lt;0.00003 )</td>
</tr>
<tr>
<td>Titanium</td>
<td>Control</td>
<td>0.25</td>
<td>0.05</td>
<td>0.18</td>
<td>0.30</td>
<td>Control/Er:YAG, ( P&lt;0.0001 )</td>
</tr>
<tr>
<td>Titanium</td>
<td>Diode</td>
<td>0.02</td>
<td>0.03</td>
<td>0.00</td>
<td>0.06</td>
<td>Diode/Er:YAG, ( P&lt;0.00003 )</td>
</tr>
<tr>
<td>Titanium</td>
<td>Er:YAG</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.001</td>
<td>Diode/Er:YAG, ( P&lt;0.00003 )</td>
</tr>
</tbody>
</table>
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.

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

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

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

Frank Schwarz, Daniel Ferrari, Christian Popovski, Jürgen Becker

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 perimplantärer Entzündungen definiert werden. 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 perimplantären Gewebestrukturen zum Implantatverlust führen.

Entstehung und Wachstum oraler Plaque-Biofilme


Schlüsselwörter
Biofilm Modell, Perimplantäre 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.


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

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


Die Adhäsion von Mikroorganismen an Oberflächen in einem wässrigen Milieu wurde in vier Phasen beschrieben⁵:

**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 Wasserstoffbrückebindung.

**Phase 4:** Colonisation und Ausbildung eines Biofilms.

Neben Streptokokken nehmen insbesondere Fusobacterium-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 spezisicher Mikroorganismen zeigten²⁰,²¹.


– Raue Oberflächen von Kronen, Implantatbautums oder Prothesenbasen akkumulieren und bewahren signifikant mehr Plaque-Biofilme als glatte Oberflächen.


– 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 vorhanden¹³. Die geringste Plaqueakkumulation zeigen hierbei polierte Implantatoberflächen (Ra = 0,03 μm), sandgestrahlte und säuregeätzte Oberflächen (/,² ε d er g e z ä t z t e n , Der g r e a t e T e s t i s t e p a r a m e t e r i n d e m B i z m i s c h l e n m ä n n i c h e n s d e m V o r h a n d e n s e i n s y s t e m i s c h e r E r k r a n k u n g e n . M i t z u n e h m e n d e m W a c h s t u m d e r M i k r o o r g a n i s m e n e n t s t e h e n z u d e m s a u e r o f f a r m e Z o n e n i n n e r h a l b d e s B i o f l m s , w e l c h e z u e i n e m m e n g e n m ä ß i g e n Z u n a h m e a n a e r o b e n B a k t e r i e n w i e z . B . V e i l l o n e l l a o r A c t i n o m y c e s s p p . f ü h r e n k a n n . W e i t e r h i n w i r d d i e i n d i v i d u e l l e P l a q u e b i l d u n g s r a t e a u c h d u r c h d a s V o r h a n d e n s e i n m e c h a n i s c h e r R e t e n t i o n s s t e l l e n s o w i e e i n e s c h l e c h t e M u n d h y g i e n e g e f ö r d e r t . B e i d e r E n t s t e h u n g p e r i i m p l a n t ä r e r I n f e k t i o n e n k o m m t i n s b e s o n d e r e r o r g a n i s m e n a n O b e r f l ä c h e n d e n B i o f l m e n e i n e ü b e r g e o r d n e t e B e d e u t u n g z u . G r u n d s ä t z l i c h k o n n t e n a u f G r u n d l a g e e x p e r i m e n t e l l e r s o w i e k l i n i s c h e n S t u d i e n n a c h f o l g e n d e E r k e n n t n i s s e g e w o n n e n w e r d e n ² ² :  

– Es besteht eine direkte Korrelation zwischen der Oberflächenrauhigkeit und den klinischen Entzündungsparametern im Bereich des marginalen Parodontiums.

Vorherrschend waren Gram-negative anaerobe sowie fakultativ anaerobe Bakterien14,15,


Es können vier verschiedene Kalziumphosphatkristallite unterschieden werden:

- \( \text{CaH}(\text{PO}_4)_2 \times 2\text{H}_2\text{O} = \text{Dikalziumphosphat-dihydrat} \) (Brushit)
- \( \text{Ca}_4(\text{PO}_4)_3(\text{HPO}_4)_2 \times 5\text{H}_2\text{O} = \text{Oktakalziumphosphat} \) – v. a. supragingival in äußeren Lagen
- \( \text{Ca}10(\text{PO}_4)_3(\text{OH})_2 = \text{Hydroxylapatit} \) – v. a. supragingival in inneren Lagen
- \( \text{[Ca}_3(\text{PO}_4)_3]_x \times \text{H}_2\text{O} = \text{Trikalziumphosphat (Whitlockite)} \) – v. a. subgingival

Mechanische Therpieansätze


Als weiterer Nachteil konventioneller Ultraschallsysteme ist neben der Hitzeentwicklung an der Arbeitsspitze bei unzureichender Kühlung3, die bei der Behandlung auftretende Aerosolbildung kritisch zu bewerten22. Weiterhin kann die überwiegend horizontal gerichtete Schwingung des Arbeitsendes einen Verlust der noch vorhandenen Restosseointegration verursachen. Demnach ist eine Verwen-

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


**Entfernung bakterieller Plaque-Biofilme – Er:YAG-Laser**

Vorhergehende In-vitro-Untersuchungen zeigten, dass eine Entfernung subgingivaler Konkremente von parodontalen erkrankten Wurzeloberflächen mit dem ERL ab einer Energiedichte von 10,6 J/cm² möglich ist24.

Um auch eine nichtchirurgische Therapie perimplantärer Infektionen an schraubenförmigen Titanimplantaten zu ermöglichen, wurde für den ERL eine spezielle kegelstumpfförmierte Faser mit axialem und radialem Strahlungsmuster entwickelt (KaVo, Biberach, Deutschland) (Abb. 2a). Als potenzieller Nachteil der radialem 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 sein38. 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 perimplantärer 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 hin35–41. 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/cm²). 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-
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 werden49. Eine Bestrahlung von SLA Implantatoberflächen konnte bis zu einer Energie von 2 Watt (25 Hz) ohne Aufschmelzungen oder strukturelle Veränderungen durchgeführt werden48 (Abb. 4).

Fazit für die Praxis

Die vergleichende Darstellung und Bewertung derzeit verfügbare Untersuchungen zur Entfernung bakterieller Plaque-Biofilme – Er, Cr:YSGG-Laser


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

**Literatur**


Removal of bacterial plaque biofilms 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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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 CO₂ 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 CO₂ 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 μm) or diode lasers (λ=810 nm) may also be of value in this field. In many other indications, the use of lasers is still experimental. Intraoperatively used photodynamic therapy or perimplant care of failing implants with the CO₂ 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), perimplant care of failing 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–aluminum–garnet laser (Nd–YAG, λ=1.064 μm), the erbium–yttrium–aluminum–garnet laser (Er–YAG, λ=2.94 μm), and the CO₂ 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
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, produce 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ laser as a cutting tool can be considered to preserve tissues 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 CO₂ 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, repertalization 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 CO₂ 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 CO₂ 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 CO₂ 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 CO2 laser resection was evaluated with the use of two different scanners (Swiflase® 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 spectrophotograph 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-tetrahydroxyphenylethylchlorin (m-THPC) [14, 15]. As a result of the irradiation, complete necroses of muscles and connective tissue were found. Nerve tissues demonstrated denyelination (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](image-url)
of Nd:YAG lasers in implant-uncovering procedures or perimplant gingival surgery should be considered inherently unsafe for such procedures.

Better results were seen with the use of a CO₂ 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 CO₂ 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 perimplantitis can be optimized using a CO₂ 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 reposition of 2 mm (±1.90 mm) after a 9.5-month observation period. However, osteointegrations were demonstrated for the first time for the CO₂ laser [6]. Most...
recent results from a study performed in beagle dogs have indicated that reossseointegration 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 intravascular 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.

References

Decontamination of rough titanium surfaces with diode lasers: microbiological findings on \textit{in vivo} grown biofilms

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 & Giamarelou 1998; Sholdone & Bartolasi 2003).

The antimicrobial activity of laser light, which depends on its photothermal effects, has been described by a number of authors in vitro (Deppe et al. 2001; Semmelhöfner et al. 2002; Kreisler et al. 2003; Soukos et al. 2003; Romanos et al. 2004) and in vivo (Mertel et al. 1997; Romanos & Nentwig 1999; Bach et al. 2000; Has et al. 2000; Deppe et al. 2001). The antimicrobial efficacy of the diode laser has been previously demonstrated in vitro (Semmelhöfner 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 intrasulcular grown biofilms on rough titanium surfaces. This study using a model of old biofilm (Anvar et al. 1994) 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 aerobic 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 Orca Laser or I.S.T., Oralis, Konstanz, and (2) 980 nm wavelength Schütz WDL 3.5, 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 each 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 (2.5 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.

Study objects and study design

Twenty-two volunteers participated in the trial. The study objects were titanium sleeves (Stoelz, 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 sandblasting before use (Alcastal 250 μm, Omnident, Rodgau Niedersößen, Germany). In order to ensure a secure intrasulcular position, the sleeves were attached in the vertical position to the buccal sides of the custom-made mandibular plastic splints (Erkohre plastic foil, 1 mm Erkodent, Pfalzgrafenweiler, Germany) with light-curing resin (Triad Gel Clear Coloreda, Dentsply, Konstanz, Germany). Figure 1a shows the top view of the splint in situ.

The splints were fitted to the patient 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 was 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 1 regular, Curaden, Kriens, Switzerland], exactly fitting in the sleeves in diameter, and with exactly 10 strokes per sleeve (Fig. 3). The swabs were placed in sterile Eppendorf tubes containing 1000 μl physiological saline according to Kite et al. (1997). The dissolved material was mixed...
on a vortexer (VF 2, Anke and Klünke, 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 to 10⁻⁴) following the method of Stülmuth et al. (1999). One hundred micro-liter aliquots of each dilution were then plated on blood agar plates (Columbia agar, Bio Mérieux, No. 43049, Marcy l'Etoile, France) and incubated under aerobic conditions at 35 ± 1°C for 24 h (Reckert 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 (Stülmuth 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 2 (control, 20 and 100) Friedman's test and for comparisons in pairs, the sign test was 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 intravenously 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: \( n.s. \) (not significant)
- control/laser 2: \( n.s. \) (not significant)

Laser 1 (810 nm wavelength) induced an average CFU reduction of 99.66% with an
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 s/100 s: $P = 0.0156$.

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 s/100 cw: $P = 0.60$.
- Laser 2 = 100 cw/1000 gw: $P = 0.61$.

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.

Discussion

Following the demonstration of the antimicrobial efficacy of diode laser light in vitro by Sennheiser-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; Prattn et al. 2003) and in vivo research (Marsh 1995; Bradshaw et al. 1997; Socransky et al. 1998, 2004; Socransky 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 2001). 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; Groszer-Schreiber et al. 2004; Kuula et al. 2004). Biofilms protect...
the colonizing microorganisms against a wide variety of exogenous influences [Anwar et al. 1992; Souli & Giamarelou 1998; Cochran et al. 2000; Sbardone & Bartolai 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. 2001]. 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 Heijdenrijk et al. [2002] basing on studies of Quirini & Listergarten [1990], Leonghardt et al. [1999] and Rosenberg et al. [1997], 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. 2003; Lencardo et al. 2001]. A primary colonization has been demonstrated with cocci [Shibli et al. 2003; Li et al. 2004]. Coccoid seem to pave the way for colonization with anaerobic organisms [Rams et al. 1999; Wu-Yuan et al. 1999] 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. 2003; Hultén 2002; Socransky et al. 2004], and rely on molecular biological analysis. However, some studies have demonstrated differing flora associated with periodontitis and peri-implantitis [Rams et al. 1999; Rutar et al. 2001; Lencardo et al. 2003; Lencardo 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 intraradial 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 [Coifeld et al. 1997; Haas et al. 1997; Kreisler et al. 2002a, 2002b, 2003; Sennhein-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; An-sacker et al. 2003; Esposito et al. 2004]. Rovald et al. [2000], for example, found a 6 log bacterial decrease by photosensitization and following 661 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; Sennhein-Kirchner et al. 2003; 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 [Romano et al. 2000; Kreisler et al. 2002]. One would, therefore, expect no risks from its clinical application [Goharkhay et al. 1999; Kreisler et al. 2002b, Romano 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,
cost predominate in biofilm formation, especially at the beginning as Leonardt 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 intracranially 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 aerobie bacteria on rough titanium surfaces although protected by accumulation in intracranially grown biofilms. Compared with the mean bacterial counts of untreated controls (40% reduction), laser irradiation treatment reduced the mean bacterial counts in the range 95.86%–99.94%. 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.

References


