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

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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 99.55% 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 94.67–99.39%.

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 90%. The actual extent of reduction was dependent on the bacteria species as well as on the irradiation mode.

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. 1995; George et al. 1996; 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. 1993; Larsen & Feldt 1996,
The antimicrobial activity of laser light, which depends on its photothermal effects, has been described by a number of authors in vitro (Depe et al. 2001; Semnchen-Kirschner et al. 2002; Kreisler et al. 2003; Srukos et al. 2003; Romanos et al. 2004) and in vivo (Müritz et al. 1997; Romanos & Nentwig 1999, Bach et al. 2000; Haus et al. 2000; Depe et al. 2001). The antimicrobial efficacy of the diode lasers has been previously demonstrated in vitro (Semnchen-Kirschner 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 intrurally grown biofilms on rough titanium surfaces. This study used a model of ‘old’ biofilm (Anwar et al. 1993) 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 anaerobic 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., 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 2 W, 600 μm wave guide fiber for 30 s.
2. 810 nm wavelength, cw mode with 2 W, 600 μm fiber for 30 s repeated five times with a 30 s pause after each 30 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.

Study objects and study design

Twenty-two volunteers participated in the trial. The study objects were titanium sleeves (Scanco, Hamburg, Germany), normally used as drill guide for dental implantology (outside diameter of 3 mm, inside diameter of 2.35, 3 mm long) which were sand-blasted before use (Alcostrahl 150 μm, Omi Dent, Rodgau, Niederlohn, Germany). To ensure a secure intraratal position, the sleeves were attached in vertical position to the buccal sides of custom-made mandibular plastic splints (Erkoder resina 800, 220 μm, Erkoder, Konstanz, Germany) with light-curing resin (Triad Gel Clear Colodex, Dentoly, Konstanz, Germany). Figure 1a shows the top view of 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 volunteer participant carried at least three sleeves in his mouth.

One titanium sleeve from each splint was left unirradiated 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 (Cuprofix CPS 12 regular, Cusden, Krifters, 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 Kitte et al. [1997]. The dissolved material was mixed
on a vortexor (VF 2, Anke and Kimmke, Stade, Br., Germany) for 1 min. For the
determination of the bacterial concentration of the different samples, the dissolved mate-
rial was serial diluted in physiological saline (10^{-1}-10^{-4}) following the method of
Stillemuth et al. [1999]. One hundred micro-
liter aliquots of each dilution were then
placed on blood agar plates (Columbia agar,
BIO Merieux, No. 43049, Marcy L'Etoile,
France) and incubated under aerobic condi-
tions at 35 ± 1°C for 24 h [Resch-In
Incubator, Forma Scientific, Mariemont, CT,
USA]. Colony-forming units (CFU) were counted
by a colony counter (Bio, Kobe, Japan).
The CFU were analyzed for morpholog-
ical differences on the agar plates and were
first classified by Gram staining [Stillemuth
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 cal-
culated for each program separately and
statistical analysis was performed as fol-
ows: In order to compare the paired ob-
servations of the modes of laser 1 (control,
20 and 100 P) Friedman's test and for com-
parisons in pain the sign test were applied
using the closure principle to adjust for
multiple testing. For the independent ob-
servations of laser 2, a non-parametric
ANOVA with two fixed factors [laser
mode and group] was used. Again compar-
isons in pairs were adjusted for multiple
testing using the closure principle.

Results
All laser irradiation techniques used in this
investigation had marked antimicrobial ef-
fects on the detected bacteria gained from
an introrally grown biofilm when com-
pared to the controls (Fig. 3a, b). The re-
duction rates were statistically significant.
- control/laser 1: P < 0.0001,
- control/laser 2 cw: P < 0.0005,
- control/laser 2 p - 100p: P < 0.001.

Laser 2 (1060 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 99.96% (3.34 log_{10} steps). The increase in CFU reduction by the repetition of irradiation was statistically significant, \( P = 0.0126 \); Friedman's test, followed by sign test (Fig. 4, b).

- \( \text{laser 1} - 20/ - 100 \): \( P = 0.0126 \).

Laser 2 (980 nm wavelength applied for 20 s at 1 W) induced an average CFU reduction of 99.57% with a ratio of 2.89 log_{10} steps. Increasing the application time to five times 20 s showed an average CFU reduction of 59.35%, 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:

- \( \text{laser 2} - 20/-100 \text{cw} : P = 0.60 \),
- \( \text{laser 2} - 100 \text{cw} / -1000 \text{pp} : P = 0.61 \).

Various species of staphyloccoci 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.

- \( \text{Staphylococcus aureus} \) (five cases) in combination with \( \text{S. lentus} \), \( \text{Streptococcus pneumoniae} \) and \( \text{Str. vestibularis} \),
- \( \text{S. crista} \) (three cases), once combined with \( \text{Str. pneumoniae} \),
- \( \text{Str. pneumoniae} \) (five cases) in combination with \( \text{Lactococcus lactis} \), \( \text{S. aureus} \), \( \text{S. crista} \) and \( \text{Str. vestibularis} \),
- \( \text{Str. sanguis} \) (in two cases) and with \( \text{S. auricularis} \) and \( \text{S. haemolyticae} \),
- \( \text{Aerococcus urinae} \) (in four cases) with \( \text{Micrococcus lyticus} \), \( \text{L. lactis} \) and \( \text{Str. pneumoniae} \),
- \( \text{L. lactis} \) (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, staphyloccoci 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 \emph{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 \emph{in vivo} grown biofilms. Biofilms have been characterized by \emph{in vitro} (Xu et al. 2000, Donlan & Costerton 2000, Pratten et al. 2003) and \emph{in vivo} research (Biedebach et al. 1999; Bradshaw et al. 1997; Smoranksy et al. 1998, 2004; Soukko 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 introral surfaces, particularly rough structures, such as the surface of implants (Krekeler et al. 1999; Marsh 1995; Bollen et al. 1996; Lee et al. 1997; Monbaliu & Lang 1998; Groesner-Schreiber et al. 2004; Kralj 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).

<table>
<thead>
<tr>
<th>Species</th>
<th>Laser 1 = 20</th>
<th>Laser 1 = 100</th>
<th>Laser 2 = 20</th>
<th>Laser 2 = 100</th>
<th>Laser 2; 100 P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strep</td>
<td>92.4-106.5</td>
<td>99.99-100</td>
<td>99.96-100</td>
<td>93.6-101.6</td>
<td>94.1-105.25</td>
</tr>
<tr>
<td>Median</td>
<td>99.54</td>
<td>100</td>
<td>99.87</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>Aerococcus urinae</em> (4 P) MR</td>
<td>96.2-100.37</td>
<td>99.98-100</td>
<td>99.65-99.95</td>
<td>99.82-100</td>
<td>99.97-100</td>
</tr>
<tr>
<td>Median</td>
<td>100</td>
<td>/</td>
<td>99.52</td>
<td>100</td>
<td>99.99</td>
</tr>
<tr>
<td>Cl</td>
<td>100</td>
<td>/</td>
<td>99.94</td>
<td>100</td>
<td>99.99</td>
</tr>
<tr>
<td><em>Lactococcus lactis</em> (5 P) MR</td>
<td>96.72</td>
<td>99.95</td>
<td>100</td>
<td>100</td>
<td>99.95</td>
</tr>
<tr>
<td>Median</td>
<td>96.79</td>
<td>99.99</td>
<td>100</td>
<td>100</td>
<td>99.95</td>
</tr>
<tr>
<td>Cl</td>
<td>96.25-100.25</td>
<td>99.83-100.06</td>
<td>99.58</td>
<td>99.39</td>
<td>98.78</td>
</tr>
<tr>
<td>All bacteria (22 P) MR</td>
<td>96.78</td>
<td>99.97-99.99</td>
<td>99.6-99.98</td>
<td>98.1-100.35</td>
<td>95.67-100.72</td>
</tr>
</tbody>
</table>

P = participants.

The colonizing microorganisms against a wide variety of exogenous influences [Anwar et al. 1993; Souli & Gianarelli 1998; Cochran et al. 2000; Sbrondone & Bartola 2003; Soukos et al. 2003; Donlan & Costerton 2000] 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. 2003]. In these cases the use/disadvantage 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. (2003) basing on studies of Quiñones & Lišťar (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 excluding 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. 2005] 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 2003, 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. 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 intracoronal in various volunatary 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. 2003].

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 [Roes-Jansscher et al. 2003; Esposito et al. 2004]. Movald et al. (2000), for example, found a 6 log bacterial decrease by photosensitization and following 867 nm laser irradiation in vitro. However, the same treatment mode applied on plaque bacterial biofilm samples of periodontal affected persons leads just to 52–92% reduction which means a ≤ 2 log decrease [Soukos et al. 2003]. As was shown previously in vitro [Haas et al. 1997, Goharkhya et al. 1999; Sennhenn-Kirchner 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 [Romano et al. 2009; Kreisler et al. 2002b]. One would, therefore, expect no risks from its clinical application [Goharkhya 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,
coci predominates in biofilm formation, especially at the beginning as Leocoxst 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. 1995; Coster ton & Lewandowski 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 mode effective for the reduction of aerobic bacteria on rough titanium surfaces although protected by accumulation in intraoral grown biofilms. Compared with the mean bacterial counts of untreated controls (0% reduction), laser irradiation treatment reduced the mean bacterial counts in the range 98.8%–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.

References


