Aus der Poliklinik für Zahnärztliche Chirurgie und Aufnahme

Westdeutsche Kieferklinik

Zentrum für Zahn-, Mund- und Kieferheilkunde

Heinrich Heine Universität Düsseldorf

Direktor: Univ.-Prof. Dr. J. Becker

Influence of an Erbium, Chromium doped Yttrium Scandium Gallium Garnet (Er, Cr:YSGG) laser on the re-establishment of the biocompatibility of contaminated titanium implant surfaces.

Dissertation

zur Erlangung des Grades eines Doktors der

Zahnmedizin

Der Medizinischen Fakultät der Heinrich Heine Universität

Düsseldorf

vorgelegt von

Enaas Nuesry

2006

Als Inauguraldissertation gedruckt mit Genehmigung der Medizinischen Fakultät der Heinrich- Heine-Universität Düsseldorf

gez: Univ.-Prof .Dr. med. Dr. rer. nat. Bernd Nürnberg

Dekan

Referent: Priv.-Doz. Dr. med. dent. Frank Schwarz

Korreferent: Prof. Dr. med. dent. Stefan Zimmer

Dedicated to my Parents

List of Abbreviations

CIS	clean implant surface areas
ERCL	Er,Cr:YSGG laser
IPB	mean initial plaque biofilm areas
MA	mitochondrial activity of cells
MP	machine polished
RPB	mean residual plaque biofilm areas
SLA	sand-blasted and acid etched
TPS	titanium plasma flamed
UC	untreated control group
SEM	scanning electron microscope

Contents

Zusammenfassung 6

Abstract 7

1. Introduction 8

1.1. Definition and Prevalence of Peri-implant Infections 8

1.2. Potential use of lasers to control disease progression 13

1.3. Treatment of Peri-implant infections€ 17

2. Aim of the study 20

3. Material and methods 21

3.2. Part I 22

3.2.1. Titanium Discs 22

3.2.2. Cell Cultures 22

3.2.3. Cell Viability Assay 23

3.2.4. SEM Observation 24

3.3. Part II 24

3.3.1. Study Population 24

3.3.2. Intraoral Splints and Titanium Discs 25

3.3.3. Measurement of Residual Plaque Biofilm Areas 26

3.3.4. Cell Cultures 28

3.3.5. Cell Viability Assay 28

3.4. Statistical Analysis 28

4. Results 29

4.1. Part I 29

4.1.1. Cell viability 29

4.1.2. SEM Observation 30

4.2. Part II 35

4.2.1. Residual Plaque Biofilm Areas 35

4.2.2. Cell viability 42

5. Discussion 43

6. References 53

Zusammenfassung

Einfluss eines Er,Cr:YSGG Lasers auf die Wiederherstellung der Biokompatibilität von kontaminierten Titanimplantat-Oberflächen

Hintergrund:
Das Ziel der vorliegenden Studie war es, den Einfluss eines Er,Cr:YSGG Lasers (ERCL) auf i) die Oberflächenstruktur/Biokompatibilität der Titanimplantate und ii) die Entfernung des Plaque-Biofilm/Wiederherstellung der Biokompatibilität der kontaminierten Titanimplantat-Oberflächen zu untersuchen.
Methode:
Intraorale Gebiss-Schienen wurden verwendet um den supragingivaler Biofilm auf gesandstrahlten und säuregeätzten Titanplattchen in vivo über einen Zeitraum von 24 Stunden zu sammeln. Anschließend wurden die n=124 Titanplättchen mit dem ERCL zufällig bestrahlt mittels einer kegelförmigen Glasfaserspitze in der Geräteeinstellung „contact mode“ und mit einer Leistung von 0.5, 1.0, 1.5, 2.0, 2.5 W bei 20 und 25 Hz. Als Kontrolle dienten unbestrahlte Titanplättchen. Alle Plättchen wurden nach der Laserbehandlung autoklaviert und mit SAOS-2 Zellen für 6 Tage inkubiert. Es wurden die folgenden Parameter beurteilt: Behandlungszeit (T), restliche Plaque-Biofilm Areale (RPB) in %, mitochondrielle Zellaktivität (MA) in Zerfälle/sec und die Zellmorphologie/Oberflächenveränderungen am Rasterelektronenmikroskop (REM).
Ergebnisse:
Die statistische Analyse innerhalb und zwischen den Gruppen ergab folgende Mittelwerte mit Standardabweichungen: RPB Areale (25 Hz): 0.5W (53.8±2.2)>1.0W (49.3±5.8)>1.5W (29.3±7.5)>2.0W (22.3±6.8)> 2.5W (9.8±6.2); MA (25 Hz): UC (2.686.285±370.678) = 0.5W (2.494.456±360.412) = 1.0 W(2.945.815±566.035) = 1.5W (2.311.019±652.454) =2.0 W(2.718.302±624.069)> 2.5W (1.825.257±373.146).
Die REM-Untersuchung konnte keine erkennbaren morphologischen Unterschiede zwischen den gelaserten und nicht-gelaserten Titanoberflächen zeigen.
Schlussfolgerung:
Im Rahmen der vorliegenden Studie konnte gezeigt werden, dass obwohl der ERCL energieabhängig eine hohe Effizienz bei der Entfernung des initialen Plaque-Biofilms aufweist, es trotzdem zu keiner Wiederherstellung der Biokompatibilität der dekontaminierten Titanoberflächen kommt.

Abstract

Background: The aim of the present study was to evaluate the influence of an Er,Cr:YSGG laser (ERCL) on i) the surface structure/ biocompatibility of titanium implants, and ii) the removal of plaque biofilms/ re-establishment of the biocompatibility of contaminated titanium surfaces.

Methods: Intraoral splints were used to collect an *in vivo* supragingival biofilm on sand-blasted and acid-etched titanium discs for 24 h. ERCL was used at an energy output of 0.5, 1.0, 1.5, 2.0, and 2.5 W for the irradiation of i) non-contaminated (20 and 25 Hz), and ii) plaque-contaminated (25 Hz) titanium discs. Non-irradiated, sterile titanium discs served as controls (UC). Specimens were incubated with SaOs-2 osteoblasts for 6 days. Treatment time, residual plaque biofilm (RPB) areas (%), mitochondrial cell activity (MA) (counts/second) and cell morphology/surface changes using scanning electrone microscopy (SEM) were assessed.

Results: i) ERCL using either 0.5, 1.0, 1.5, 2.0, or 2.5 W at both 20 and 25 Hz resulted in comparable mean MA values as measured in the UC group. A monolayer of flattened SaOs-2 cells, showing complete cytoplasmic extensions and lamellopodia was observed in both ERCL and UC groups. However, mean MA values were significantly higher in the UC group ii) mean RPB areas decreased significantly with increasing energy settings (53.8±2.2 at 0.5 W to 9.8±6.2 at 2.5 W)..

Conclusions: Within the limits of the present study, it was concluded that even though ERCL exhibited a high efficiency to remove plaque biofilms in an energy dependent manner, it failed to re-establish the biocompatibility of contaminated titanium surfaces.

1. Introduction

1.1. Definition and Prevalence of Peri-implant Infections

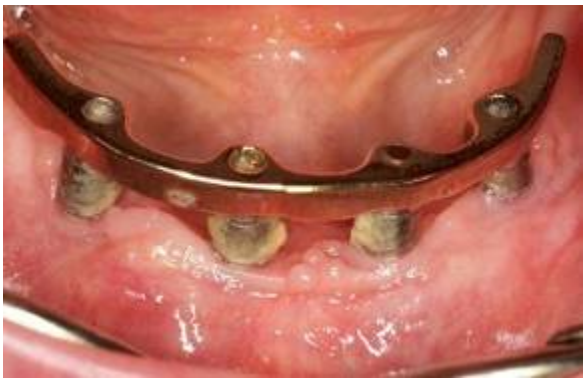
These days as dental implants are gaining more importance as a successful method for oral rehabilitation, it has also drawn an increasing attention to the subsequent problems that arise following their insertion. In particular, there is considerable evidence supporting the cause- and effect- relationship between microbial plaque colonization and the pathogenesis of implant failures (Albrektsson and Isidor, 1994; Alcoforado et al., 1991; Becker et al., 1990; Mombelli et al., 1988). Nowadays, the term peri-implant disease is collectively used to describe biological complications in implant dentistry including peri-implant mucositis and peri-implantitis. While, peri-implant mucositis includes reversible inflammatory reactions located solely in the mucosa adjacent to an implant, peri-implantitis was defined as an inflammatory process that affects all tissues around an osseointegrated implant in function resulting in a loss of the supporting alveolar bone (Albrektsson and Isidor, 1994).

The prevalence of peri-implantitis is difficult to estimate since the criteria determining the implant success are not uniform (Albrektsson et al., 1986; Buser et al., 1990; van Steenberghe, 1997). However, considering clinical and radiological threshold parameters assessed at different implant designs, it may vary between 10% and 29% (Brägger et al., 1996; Buser et al., 1997; Karoussis et al., 2003; Karoussis et al., 2004). Moreover, recent findings from a multicenter study including 159 patients and 558 implants revealed that during the second and third year as many as 2% of the remaining implants failed, and failure occurred more frequently in subjects with a high degree of plaque accumulation (van Steenberghe et al., 1993).

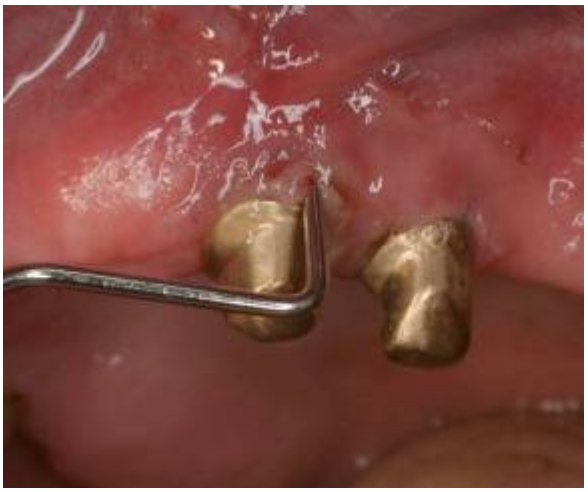
Since peri-implantitis was also classified as a disease process associated with microorganisms related to chronic periodontitis (Leonhardt et al., 1999; Mombelli et al., 1987; Rams et al.,

1991), it was assumed that the removal of bacterial plaque biofilms may also be a prerequisite for treatment of peri-implant infections (Fig. 1).

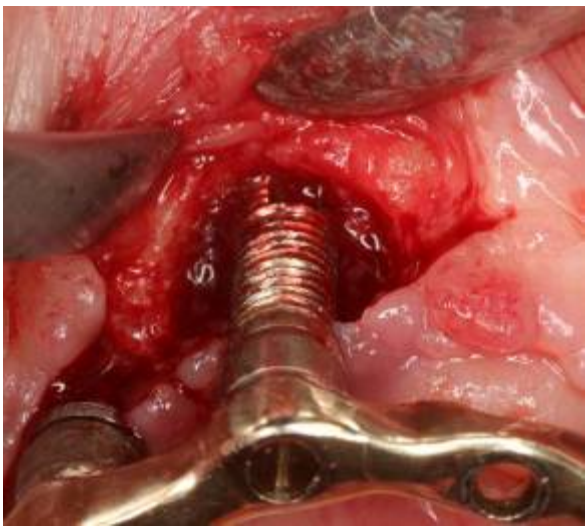
Fig. 1

a. 

Enhanced plaque accumulation at the suprastructure of endosseous titanium implants

b. 

Clinical signs of acute Peri-implantitis: Bleeding on Probing and pus formation

c. 

Circumferential bone loss as a result of an ongoing disease progression

As a consequence of the fact that rough surfaces accumulate and retain more plaque than smooth surfaces, nowadays, most implant systems use highly polished titanium parts for the transmucosal abutment connection (Bollen et al., 1997; Quirynen et al., 1993; Rimondini et al., 1997; Siegrist et al., 1991; Zitzmann et al., 2002). On the other hand, however, there is also considerable evidence supporting the view that a certain degree of surface roughness is needed for an optimal marginal soft tissue integration, to support the establishment of an effective seal between the oral environment and the endosseous part of a titanium implant (Bollen et al., 1996; Lindhe and Berglundh, 1998; Quirynen et al., 1996).

Accordingly, in recent years, extensive research has been performed in order to investigate the biological soft tissue seal at different types, materials and roughness of dental implants (Abrahamsson et al., 1996; Abrahamsson et al., 1998; Abrahamsson et al., 2002). In particular, it was observed that abutments made of commercially pure titanium or highly sintered Al_2O_3 ceramic allowed the formation of a mucosal attachment which included one epithelial and one connective tissue portion (Abrahamsson et al., 1998). However, it was also demonstrated that the attachment between the peri-implant mucosa and titanium abutments with either a turned or acid-etched surface was similar from both a quantitative and a qualitative aspect (Abrahamsson et al., 2002).

In general, the transmucosal attachment is comprised of a barrier epithelium and a zone of connective tissue attachment. Close attention has been paid to the implant/ subepithelial connective tissue interface, since this zone of interaction is apparently not recognized as a wound tissue and therefore does not call for an epithelial lining (Berglundh et al., 1991; Berglundh and Lindhe, 1996; Fartash et al., 1990). At both machined and rough surfaces, the subepithelial connective tissue is located between the apical part of the barrier epithelium and the implant supporting alveolar bone and can be divided into two different zones. In

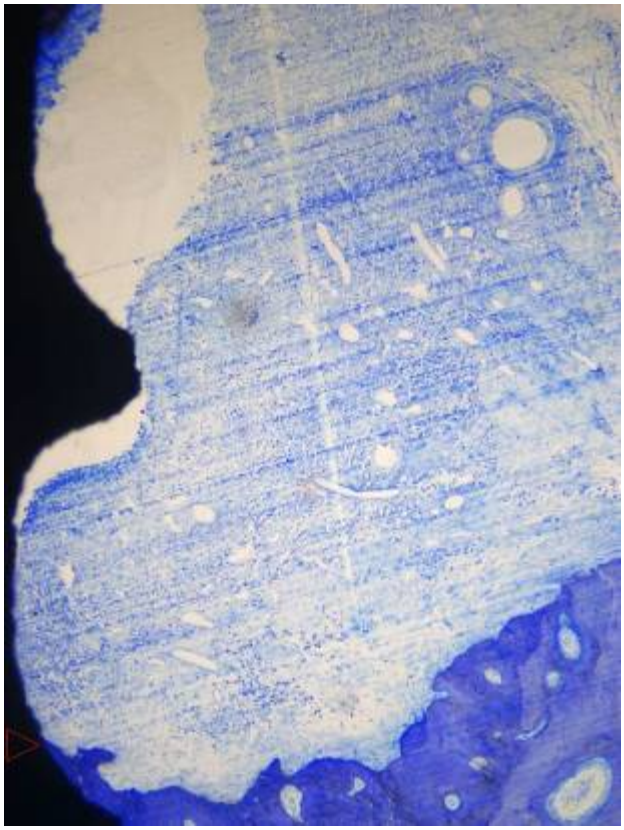
particular, the inner zone has been described to be poorly vascularized, consisting of numerous dense collagen fibers, running close to the implant surface predominantly in a parallel direction (Abrahamsson et al., 1996; Berglundh et al., 1994; Buser et al., 1992; Cochran et al., 1997; Gotfredsen et al., 1991). The outer zone, however, appeared to be formed of fibers running in different directions, richer in cells and blood vessels (Buser et al., 1992). In the past years, several modifications of specific surface properties such as topography, structure, chemistry, surface charge, and wettability have been investigated in order to improve marginal soft tissue integration at different implants (Albrektsson, 1983).

Even though a direct connective tissue contact has been observed for smoothly polished, roughly sandblasted and plasma-sprayed implant surfaces, the collagen fibers were parallel oriented without showing any signs of perpendicular insertion to the respective implant surfaces (Buser et al., 1992; Listgarten et al., 1992). However, the lack of perpendicular insertion of collagen fibers at non-submerged or two-stage implants might also be influenced by uncontrollable plaque accumulation and subsequent bacterial contamination of the internal portion of implants, leading to an inflammatory cell infiltrate in the peri-implant mucosa (Quirynen and van Steenberghe, 1993; Quirynen et al., 1994).

Chronic periodontitis is defined as a destruction of the tooth supporting structures including the periodontal ligament, bone and soft tissues, which in turn may result in tooth loss (Kinane, 2001). Similarly, the host response to biofilm formation on the surface of titanium implants includes a series of inflammatory reactions which initially occur in the soft tissue but which may subsequently progress and lead to a loss of the supporting alveolar bone. The response of the gingiva and the peri-implant mucosa to early and more long-standing periods of plaque formation was analyzed both in experimental animal (Berglundh et al., 1992; Ericsson et al., 1992) and human clinical trials (Pontoriero et al., 1994). During the course of the study, it was

observed that similar amounts of plaque formed on the tooth and implant segments of the dog dentition. The composition of both developing plaques was also similar. In this model, peri-implantitis lesions were induced by terminating the plaque control regimen, and placement of cotton ligatures submarginally around implants (Baron et al., 2000; Lang et al., 1993; Lindhe et al., 1992; Marinello et al., 1995; Schou et al., 1993; Zitzmann et al., 2004) (Fig. 2).

Fig. 2



Histopathological signs of a chronic peri-implant infection showing a mixed inflammatory cell infiltrate (Toluidine blue stain, original magnification x 150, dog model)

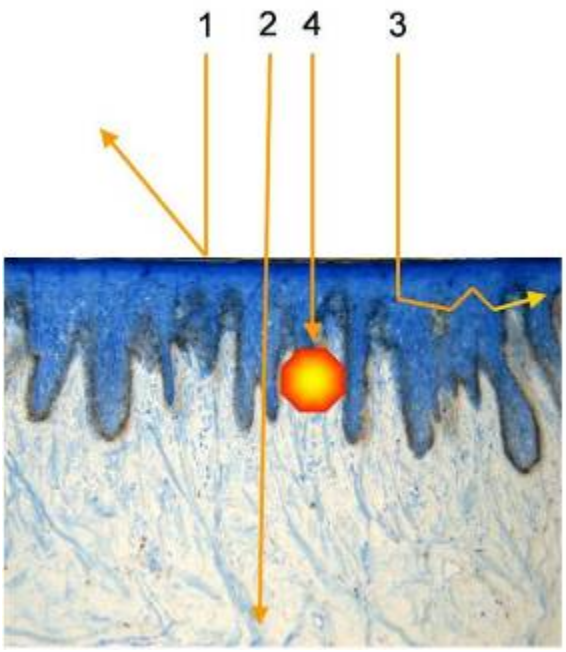
1.2. Potential use of lasers to control disease progression

A major goal of periodontal treatment is to resolve inflammation and thereby arrest disease progression (Caffesse et al., 1995). Ideally, periodontal therapy does not include arresting the disease only but also regeneration of the tissues which have been lost due to disease. This includes *de novo* formation of connective tissue attachment and the regrowth of alveolar bone (Caton and Greenstein, 1993). The results from controlled clinical studies have shown that nonsurgical treatment (*i.e.*, scaling and root planing using hand instruments) and various types of conventional surgical treatment may lead to a clinically important and statistically significant probing pocket depth reduction and clinical attachment level gain (Isidor and Karring, 1986; Kaldahl et al., 1996; Ramfjord et al., 1987). However, histological studies demonstrated that healing following nonsurgical and conventional surgical periodontal therapy is mainly characterized by formation of a long junctional epithelium along the instrumented root surfaces and no predictable regeneration of attachment apparatus was detected (Aukhil et al., 1988; Bowers et al., 1989; Caton et al., 1980; Caton and Greenstein, 1993; Sculean et al., 2000; Sculean et al., 2003). In this context, the formation of a smear layer after both mechanical scaling and root planing and ultrasonic instrumentation has been reported to be detrimental to periodontal tissue healing as it may inhibit reattachment of cells to the root surface (Blomlöf and Lindskog, 1995; Blomlöf et al., 1997). However, additional root surface conditioning with various substances such as ethylenediaminetetraacetic acid gel (EDTA) at neutral pH, citric- and ortho-phosphoric acids has been shown to be effective in removing the smear layer and exposing the collagenous matrix of dentin (Blomlöf and Lindskog, 1995; Blomlöf et al., 1997; Blomlöf et al., 1996; Polson et al., 1984).

In recent years, the use of laser radiation has been expected to serve as an alternative or adjunctive treatment to conventional, and mechanical periodontal therapy. Various advantageous characteristics, such as hemostatic effects, selective calculus ablation or

bactericidal effects against periodontopathic pathogens might lead to improved treatment outcomes (Ando et al., 1996; Aoki et al., 1994; Folwaczny et al., 2002b). The wavelengths of the lasers most commonly used in periodontics, which include diode lasers, the Nd:YAG laser (neodymium-doped: yttrium, aluminium and garnet), the Er:YAG laser (erbium-doped: yttrium, aluminium and garnet) and the CO_2 (carbon-dioxide) laser, range from 819 nm to 10,600 nm. Due to an excellent soft tissue ablation capacity, CO_2 lasers have been successfully used as an adjunctive tool to deepithelialize the mucoperiosteal flap during traditonal flap surgery (Centty et al., 1997). Diode and Nd:YAG lasers were mainly used for laser-assisted subgingival curettage and disinfection of the periodontal pocket with various degrees of success (Cobb et al., 1992; Liu et al., 1999; Moritz et al., 1998). However, several studies reported the thermal side effects, such as melting, cracking or carbonization when CO_2 and Nd:YAG lasers were used directly on root surfaces (Israel et al., 1997; Tewfik et al., 1994; Tucker et al., 1996; Wilder-Smith et al., 1995). In case of the CO_2 laser these negative effects could be avoided when irradiation was performed in a pulsed mode with a defocused beam (Barone et al., 2002). So far, there is limited information about the effects of diode laser radiation on the surface properties of root surfaces. The results from recent studies showed that this laser may also cause damage to periodontal hard tissues if irradiation parameters are not adequate (Kreisler et al., 2002a; Schwarz et al., 2003e). Furthermore, neither CO_2 nor Nd:YAG nor diode lasers were effective in removing calculus from the root surface (Liu et al., 1999; Moritz et al., 1998; Tucker et al., 1996). Since, according to the cause-related concept of periodontal therapy, the main objective of treatment is to remove all calcified deposits from the root surface (O'Leary, 1986), these types of lasers should only be used as an adjunct to mechanical periodontal treatment (Fig. 3).

Fig. 3



Laser Tissue Interactions:

1 Reflection

2 Transmission

3 Scattering

4 Absorption

Photothermal Effects

Fluorescence

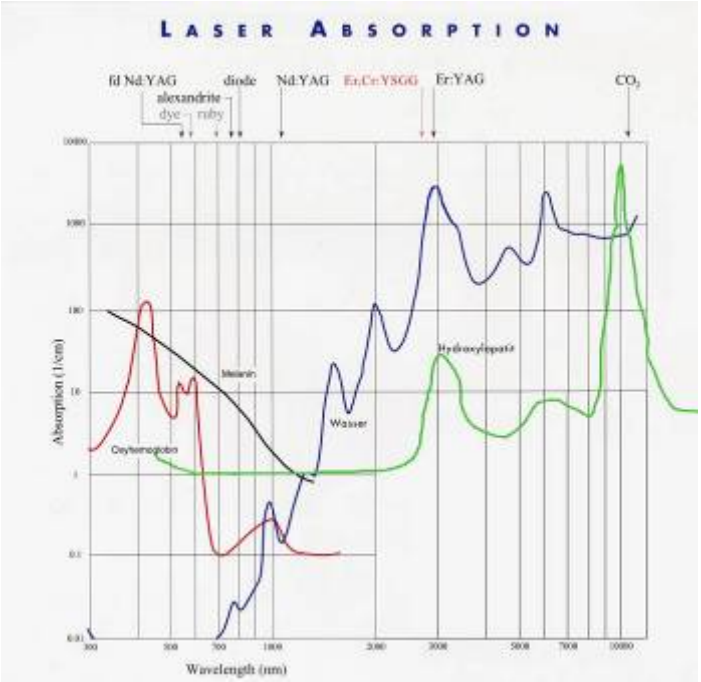
Heat

On the other hand, close attention has been paid to the clinical applicability of the Er:YAG laser with the wavelength of 2.94 µm in the near infrared spectrum. Because of the high absorption of its emission wavelength by water, this laser system provides a capability to effectively remove calculus from periodontally diseased root surfaces without causing thermal side effects to the adjacent tissue (Aoki et al., 1994; Eberhard et al., 2003; Schwarz et al., 2003e). Furthermore, several studies have reported antimicrobial effects against periodontopathic bacteria and the removal of lipopolysaccharides by Er:YAG laser radiation (Ando et al., 1996; Folwaczny et al., 2002b; Folwaczny et al., 2003; Sugi et al., 1998; Yamaguchi et al., 1997). The absence of thermal damages was most likely due to the optical characteristics of its wavelength of 2940 nm, since the Er:YAG laser theoretically has a 10 and 15,000 – 20,000 times higher absorption coefficient of water than the CO_2 and the Nd:YAG lasers, respectively (Hale and Querry, 1973; Robertson and Williams, 1971). Another important observation was the lack of a smear layer formation on the root surface

after Er:YAG laser instrumentation (Schwarz et al., 2003e; Theodoro et al., 2003). However, histological and SEM examination showed that under *in vitro* conditions the Er:YAG laser ablated not only the calculus, but also a certain amount of the superficial portion of the underlying cementum (Aoki et al., 1994; Folwaczny et al., 2000; Israel et al., 1997). In contrast, recently published studies reported a lack of cementum removal when laser instrumentation was performed under *in vivo* conditions (Eberhard et al., 2003; Schwarz et al., 2003e). Controlled clinical trials (Schwarz et al., 2001; Schwarz et al., 2003c; d) and case report studies (Schwarz et al., 2000; Watanabe et al., 1996) have indicated that nonsurgical periodontal treatment with an Er:YAG laser may lead to significant clinical improvements as evidenced by probing depth reduction and gain of clinical attachment. These improvements were comparable to those obtained following treatment with hand instruments. Furthermore, the obtained clinical results were maintained for a period of 2 years (Schwarz et al., 2003c). Preliminary clinical results have also indicated that this minimally invasive device may allow instrumentation of very deep and narrow pockets without leading to major trauma of the hard and soft tissues; *i.e.* removal of tooth substance and increase of gingival recession (Schwarz et al., 2001). Most recently, clinical results have also demonstrated that treatment of deep intrabony periodontal defects with the use of an Er:YAG laser alone or in combination with the application of an enamel matrix protein derivative may lead to a clinically important and statistically significant gain of clinical attachment (Schwarz et al., 2003f; Sculean et al., 2004). In order to avoid any damages to the root surface, a subgingival calculus detection system with fluorescence induced by 655 nm InGaAsP (indium-gallium-arsenide-phosphate) diode laser radiation has been recently included in an Er:YAG laser device. Preliminary *in vitro* results have shown that 655 nm diode laser radiation induces significantly stronger fluorescence in subgingival calculus than in cementum, suggesting that calculus removal may be selectively performed (Folwaczny et al., 2002a; Krause et al., 2003). Thus, all the available

data taken together seem to indicate that laser wavelengths in the range of 3,000 nm may possess some qualities which could also support healing of peri-implant infections (Fig. 4).

Fig. 4



Absorptions characteristics of different wavelengths in biological tissues

1.3. Treatment of peri-implant infections

In recent years, several conventional treatment approaches including plastic curettes, sonic/ultrasonic scalers and air-powder flow have been recommended for the debridement of contaminated implant surfaces (Augthun et al., 1998; Fox et al., 1990; Matarasso et al., 1996; Ruhling et al., 1994). However, as these conventional tools were observed to be insufficient in the elimination of bacteria on rough implant surfaces (Schwarz et al., 2005b; Schwarz et al., 2006a), adjunctive chemical agents (i.e. irrigation with local disinfectants, local/ or systemic antibiotic therapy) were examined clinically and proved to enhance healing following treatment (Ericsson et al., 1996; Mombelli and Lang, 1992; Schenk et al., 1997). Even though air-powder flow was also successfully used for decontamination of implant surfaces *in vitro*,

its use seems to be limited because of surface changes (Kreisler et al., 2005) and an increased risk of emphysema (Van de Velde et al., 1991).

In addition to these conventional tools, regenerative treatment procedures have been advocated for the restoration of the implant supporting tissues (Ericsson et al., 1996; Hürzeler et al., 1997; Nociti et al., 2001; Persson et al., 1996; Schou et al., 2003a; b; Schou et al., 2003c; Schou et al., 2004). Most of these studies employed the concept of guided bone regeneration (GBR) which involves the placement of a barrier membrane to protect the blood clot and create a secluded space around the bone defect enabling bone regeneration without competition from other tissues (Dahlin et al., 1988). In recent years, a variety of different membrane materials have been successfully used for GBR procedures, ranging from non-resorbable materials such as expanded polytetrafluorethylene (e-PTFE) to bioresorbable membranes composed of dura-mater, polylactic acid, polyglycolic acid and polyurethane (Greenstein and Caton, 1993; Hardwick et al., 1994; Hutmacher et al., 1996; Kohal et al., 1998; Magnusson et al., 1988). Most recently, many investigations focused on the use of products derived from type I and type III porcine or bovine collagen (for review see Bunyaratavej and Wang, 2001). Some advantageous properties of collagen over other materials include hemostatic function allowing an early wound stabilization, chemotactic properties to attract fibroblasts, and semipermeability, thus facilitating nutritient transfer (Postlethwaite et al., 1978; Schwarz et al., 2006b; Yaffe et al., 1984). However, a major drawback of native collagen is the fast biodegradation by the enzymatic activity of macrophages, polymorphonuclear leucocytes and periodontopathic bacteria resulting in a poor membrane resistance to collapse allowing undesirable cell types to enter the secluded wound area (Rothamel et al., 2004; Sela et al., 2003; Tatakis et al., 1999). However, the collapse may be prevented by means of implantation of bone grafts or bone graft substitutes into the defect to support the membrane preserving its original position. Even though several treatment

approaches have been indeed demonstrated beneficial clinical and radiological effects, the amount of documented bone regeneration and re-osseointegration, as observed histologically in animals, varied considerably (Ericsson et al., 1996; Hürzeler et al., 1997; Nociti et al., 2001; Persson et al., 1996; Schou et al., 2003a; b; Schou et al., 2003c; Schou et al., 2004). In most of these studies, the re-establishment of osseointegration has even been questioned (Schou et al., 2004). Several factors have been discussed to explain the lack of re-osseointegration at contaminated implants. In particular, recent studies have demonstrated that plaque biofilms may alter the surface characteristics of titanium implants. It was presumed that bacterial contamination of a titanium surface may affect its dioxide layer resulting in a lower surface energy and subsequently reduced tissue integration (Baier and Meyer, 1988; Sennerby and Lekholm, 1993).

In order to overcome some of these problems encountered for conventional treatment procedures, the use of different laser systems has also been purposed for treating peri-implant infections. As lasers can perform excellent tissue ablation with high bactericidal and detoxification effects, they are expected to be one of the most promising new technical modalities for treatment of failing implants (Kato et al., 1998; Kreisler et al., 2002d; Kreisler et al., 2005). However, the interaction between laser light and metal surfaces is mainly determined by the degree of absorption and reflection. Each metal features a certain spectral reflection capacity which is dependent on the specific wavelength of the laser. The reflection capacity of titanium for the Er:YAG laser with its wavelength of 2,940 nm in the near infrared spectrum is 71% and rises up to 96% for the CO_2 laser at 10,600 nm (Lide, 2002). Indeed, recent *in vitro* studies have demonstrated that, in an energy dependent manner, only the CO_2 (carbon-dioxide) laser, the diode laser (980 nm) and the Er:YAG (erbium-doped: yttrium, aluminium and garnet) laser may be suitable for the irradiation of implant surfaces, since the implant body temperature did not increase significantly during irradiation (Kato et al., 1998;

Kreisler et al., 2002b; Kreisler et al., 2002c; Oyster et al., 1995; Romanos et al., 2000). Since, neither CO_2 nor diode lasers were effective in removing plaque biofilms from root surfaces or titanium implants, both types of lasers were only used adjunctive to mechanical treatment procedures (Bach et al., 2000; Schwarz et al., 2003e; Tucker et al., 1996). In contrast, preliminary experimental and clinical results have shown, that an Er:YAG laser seemed to be capable of effectively removing bacterial deposits from both smooth and rough titanium implants without damaging their surfaces (Kreisler et al., 2005; Matsuyama et al., 2003; Schwarz et al., 2003b; Schwarz et al., 2005b).

Most recently, an Erbium, Chromium-doped: Yttrium-Scandium-Gallium-Garnet (Er,Cr:YSGG) laser with a wavelength of 2,780 nm, which is more highly absorbed by OH^- ions than water molecules (Featherstone, 2000) has been introduced in order to improve hard tissue ablation (Kimura et al., 2001a; Kimura et al., 2001b; Wang et al., 2005). A commercially available Er,Cr:YSGG laser device using an ablative hydrokinetic process has also been reported to have an improved performance for the removal of bacterial contaminants from implant surfaces (Miller, 2004). Until now, however, no investigations are available evaluating this device for the removal of plaque biofilms from rough titanium implants.

2. Aim of the study

The aim of the present study was to investigate

1. the effects of an Er,Cr:YSGG laser device, used with a cone-shaped application tip, on the surface structure and the biocompatibility of titanium implants (Part I), and
2. Its influence on the removal of early plaque biofilms grown on titanium implants and subsequently the re-establishment of the biocompatibility of the irradiated titanium surfaces (Part II).

3. Material and methods

3.1. Laser device

An Er, Cr: YSGG laser device[†] device (Fig. 5a) at a wavelength of 2,780 µm and a cone-shaped fibre tip (600µm, maximum power 2.5 W) emitting an axial and radial laser beam were selected for laser irradiation (Fig. 5b). In both parts of the study, irradiation was performed in soft tissue mode (S) at a setting of 50% water and 50% air.

Fig. 5

A.

Er,Cr:YSGG laser device

Wavelength: 2,780 nm
Power: 0.1 to 8.0 W
Repetition Rate: 10 to 50 Hz
Pulse Energy: 300 mJ
Laser Classification: IV

Touch Panel

b.

600µm cone-shaped fibre tip

[†] Waterlase MD, Biolase, San Clemente, CA, USA

3.2. Part I

3.2.1. Titanium Discs

A total of n=88 sand blasted and acid-etched titanium discs[‡] (0.7 cm^2, 2 mm thick) (SLA) were randomly irradiated at 0.5, 1.0, 1.5, 2.0, and 2.5 W using either 20 Hz (group a, n=40) or 25 Hz (group b, n=40). Non-irradiated discs served as control (UC) (n=8). The fibre tip was guided parallel to the titanium surfaces in contact mode. Each titanium disc was only scanned once to standardize the treatment time. The needed amount of time for irradiation was, on average, 2 minutes per titanium disc. All treatments were performed by the same experienced operator.

3.2.2. Cell Cultures

Subsequent to irradiation, a total of n=66 specimens (groups a and b, n=30 each; UC, n=6) were autoclaved and placed into 24-well plates (Fig. 6).[§] Human osteoblast like SaOs-2 cells[‖] (2 x 10^4 cells, fourth passage) were suspended in McCoy's 5A medium[¶] containing 1% penicillin/streptomycin[#] and 10% fetal bovine serum[#] and then inoculated onto the well chambers. Culturing was set at 37°C in a humified atmosphere of 95% air and 5% CO_2.

Fig. 6

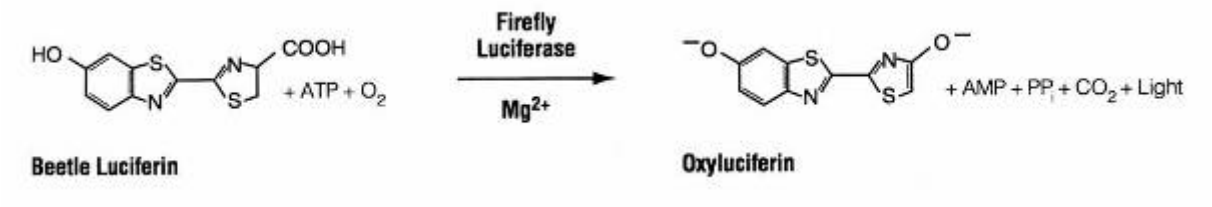
24-well plate

[‡] Promote, Altatec, Wurmberg, Germany
[§] Lap Tek Chamber Slide, Nalge Nunc, Naperville, IL, USA
[‖] ATCC, No. HTB 85, Manassas, VA, USA
[¶] MCM, Gibco No. 21017-025, Life Technologies GmbH, Karlsruhe, Germany
[#] Gibco® Invitrogen, Karlsruhe, Germany

3.2.3. Cell Viability Assay

At day 6 the mitochondrial activity (MA) in SaOs-2 osteoblasts was measured using a luminescent cell viability assay.[**] This assay quantifies the ATP present, which signals the presence of metabolic active cells and is based on the luciferase-catalyzed reaction of luciferin and ATP. In particular, mono-oxygenation of luciferin is catalyzed by luciferase in presence of Mg^{2+}, ATP and molecular oxygen (Fig. 7). 100 µl reagent was added to the wells and incubated for 10 min at room temperature. The luminescent signal was recorded for 1 sec per well in a counter.[††]

Fig. 7



Luminescent cell viability assay

The detection reagent was prepared as follows:

- the CellTiter Glo® Buffer was combined with CellTiter Glo® Substrate
- an equal volume of CellTiter-Glo® Reagent was added to each well of the cell culture medium
- after mixing for 2 minutes, the plate remained at room temperature for 10 minutes to stabilize the luminescence
- the luminescence was detected with integration time set for 0.25 to 1 second.

[**] CellTiter-Glo, Promega, Madison, WI, USA
[††] Top Count, Canberra-Packard GmbH, Dreieich, Germany

3.2.4. SEM Observation

Two titanium discs of each group (groups a and b, n=10 each; UC, n=2) were gently washed with phosphate buffered saline (PBS) to remove cells not attached to the surface and fixed for 30 minutes with 4% glutaraldehyde in PBS (pH=7.4) at room temperature and then washed in PBS for 15 min. The specimens were dehydrated in increasing concentrations of acetone (from 40 to 100%, 10% steps). After drying in hexamethyldisilazane the specimens were sputter coated with gold and examined using scanning electron microscopy (SEM).‡‡

3.3. Part II

3.3.1. Study Population

Three healthy volunteers (2 women, 1 men, mean age 30.7±2.1 years) were included in the study. Each participant was given a detailed description of the procedure and was required to sign an informed consent. The study protocol was approved by the local ethic's committee. The criteria needed for inclusion were: 1) no systemic use of antibiotics during the last 6 months, 2) good level of oral hygiene (Plaque Index <1), (Loe, 1967) (Table 1), 3) no signs of aggressive/chronic periodontitis or any inflammatory conditions of the surrounding soft tissues, and 4) non-smoker.

Table 1: Plaque Index (Loe, 1967)

Scores	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposit s within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

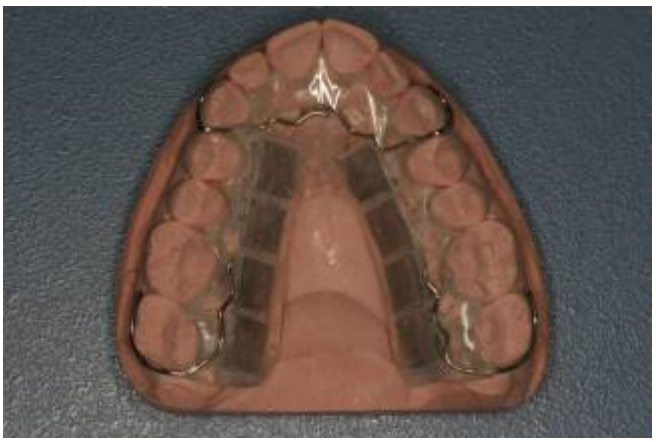
‡‡ DSM 950, Zeiss, Oberkochen, Germany

3.3.2. Intraoral Splints and Titanium Discs

Prior to the investigation, the subjects received a professional tooth cleaning. The volunteers obtained acrylic appliances for the upper jaw with eight titanium discs[‡] each to collect a supragingival plaque biofilm (Hahn et al., 1992; Schwarz et al., 2005b; Schwarz et al., 2006a). Specimens were inserted into depressions with sticky wax directed towards the palate with a space of approximately 1 mm in order to provide a nutritious aqueous environment (Fig. 8). The splints were worn by the volunteers for 24 hours (Rimondini et al., 1997; Siegrist et al., 1991). The subjects were allowed to maintain their regular diet and retained the splints intra-orally throughout the whole experimental period, except during their daily mechanical tooth brushing (only with tap water, no toothpaste or mouthrinse were allowed).

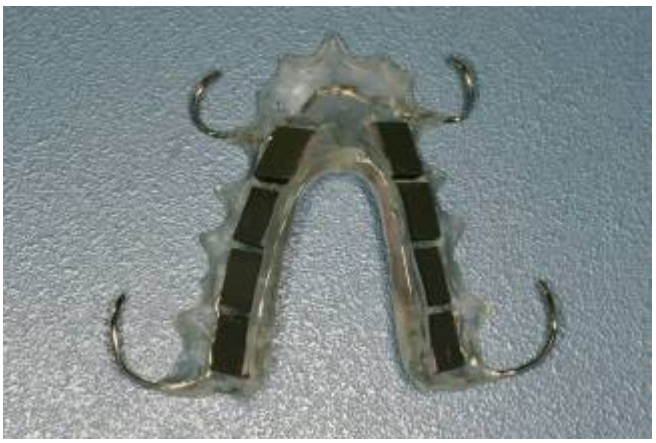
Fig. 8

a.



Removable splint showing the location of the titanium discs in the upper jaw

b.



Eight specimens were inserted into depressions with sticky wax towards the palate

Erythrosine dye was used to stain the plaque biofilm grown on the implant surfaces. Only specimens exhibiting a homogenous initial plaque biofilm (IPB), as evidenced by light microscopic observation,[§§] were included in the study.

A total of n=30 titanium discs were collected and randomly irradiated at 0.5, 1.0, 1.5, 2.0, and 2.5 W using 25 Hz. Unworn and untreated titanium discs served as control (UC) (n=6). The fibre tip was guided parallel to the titanium surfaces in contact mode. The end point of treatment was defined as the inability to remove residual plaque biofilm areas. All treatments were performed by the same experienced operator.

3.3.3. Measurement of Residual Plaque Biofilm Areas

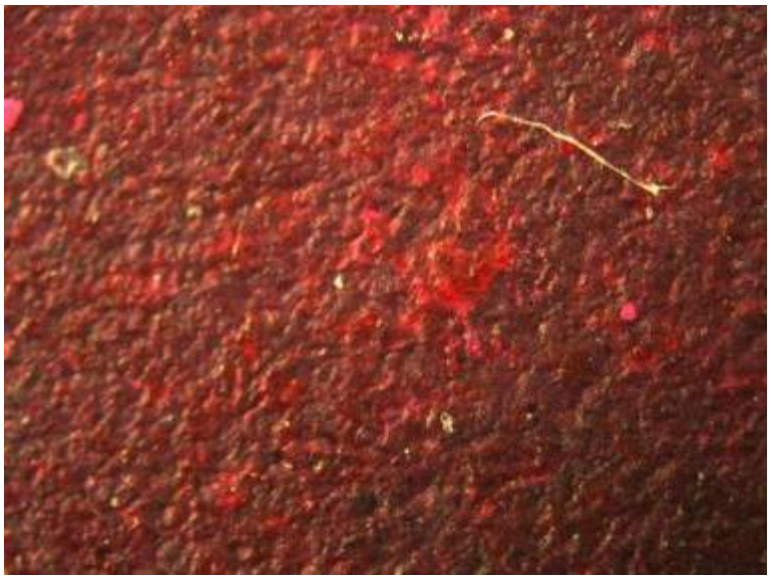
For image acquisition a digital camera[‖‖] was mounted on a binocular light microscope.[§§] Digital images (original magnification x 40) were evaluated using a software program.[¶¶] For each titanium disc, mean initial plaque biofilm (IPB) areas (Fig. 9), residual plaque biofilm (RPB) areas and clean implant surface (CIS) areas were measured as a percentage of the scanned surface at 20 fields selected at random. For calculation of RPB and CIS areas, respective IPB areas were defined as 100%. All measurements were performed by one calibrated examiner masked to the specific experimental conditions .

[§§] Olympus BX50, Olympus, Hamburg, Germany
[‖‖] ColorView III, Olympus, Hamburg, Germany
[¶¶] SIS analySIS Auto Software 3.2, Soft imaging System GmbH, Münster, Germany

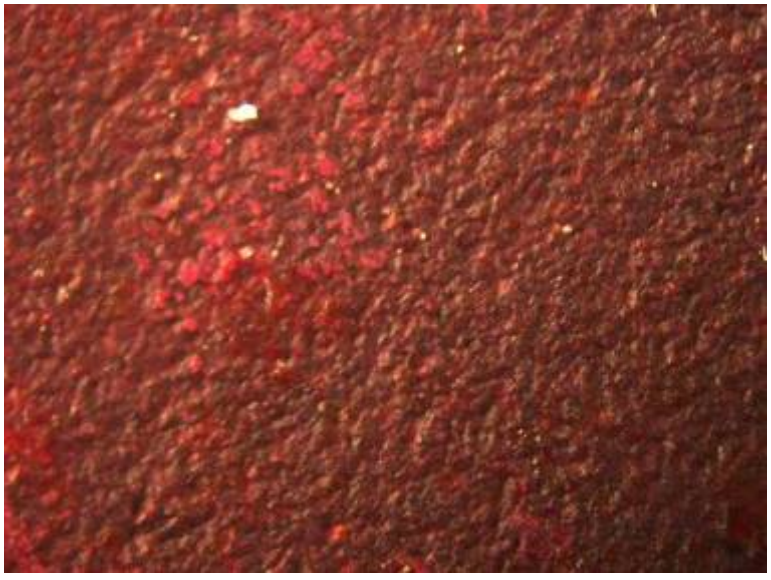
Fig. 9

a. 

Homogeneously stained Plaque Biofilm after 24 hours
IPB: 100%

b. 

IPB: 100%

c. 

IPB: 98%

3.3.4. Cell Cultures

Subsequent to measurement of RPB areas, specimens were autoclaved and incubated with SaOs-2 osteoblasts according to the procedure as described in Part I.

3.3.5. Cell Viability Assay

At day 6 MA in SaOs-2 osteoblasts was measured according to the procedure as described in Part I.

3.4. Statistical Analysis

A software package[##] was used for the statistical analysis defining titanium discs as statistical unit. Mean values and standard deviations were calculated for each group. The data rows were examined with the Kolmogorow-Smirnow test for normal distribution. Analysis of variance (ANOVA) and post-hoc testing using Bonferroni`s correction for multiple comparisons was used for comparisons within and between groups. Results were considered statistically significant at $P<0.05$.

The following parameters were included in the statistical analysis:

MA	mitochondrial activity of SaOs-2 osteoblasts	Groups a and b, UC
IPB	mean initial plaque biofilm areas	Group b, UC
RPB	mean residual plaque biofilm areas	Group b, UC
CIS	clean implant surface areas	Group b, UC

[##] SPSS 14.0, SPSS Inc., Chicago, IL, USA

4. Results

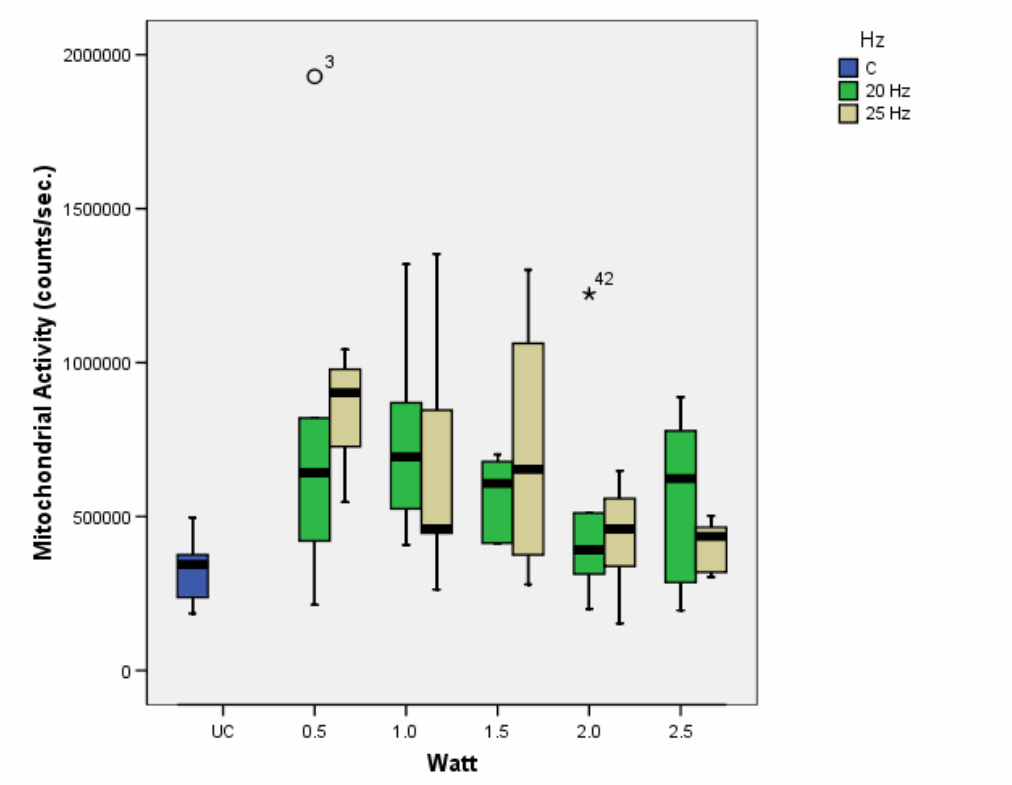
4.1. Part I

4.1.1. Cell viability

In general, during the experimental period, there were no signs of any bacterial or fungal contamination of the well chambers.

MA of SaOs-2 osteoblasts, expressed as luminescent output (counts per second), is presented in Figure 10. In particular, ERCL using either 0.5, 1.0, 1.5, 2.0, or 2.5 W at both 20 and 25 Hz resulted in comparable mean values of MA as measured in the UC group (P>0.05; respectively). Even though mean scores of MA seemed to decrease particularly at 2.0 and 2.5 W, these changes did not reach statistical significance (P>0.05; respectively) (Fig. 10).

Fig. 10



Boxplots with outliers for the medians and Q1-Q3 quartiles of mitochondrial activity of SaOs-2 osteoblasts (counts per second) at non-contaminated implant surfaces (Part I) in different groups.

4.1.2. SEM Observation

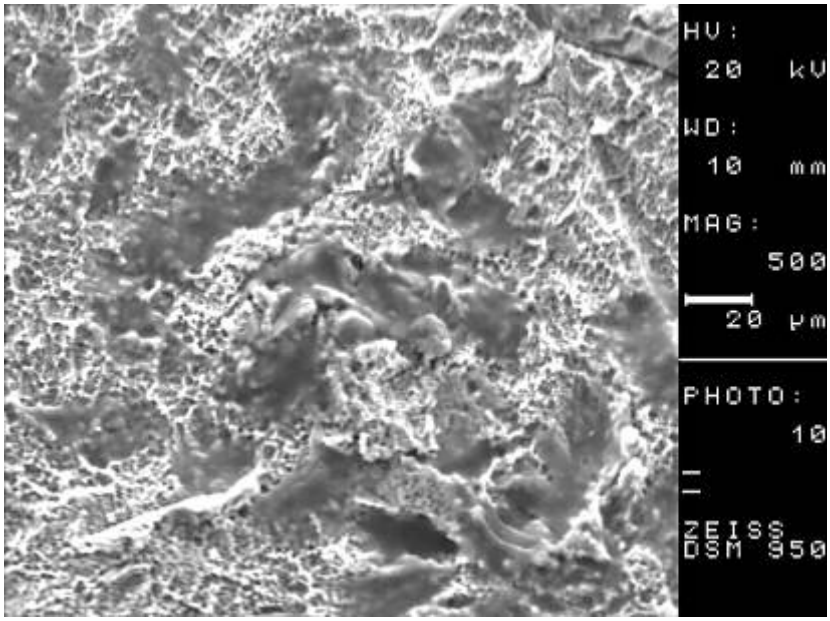
After 6 days following incubation, SEM observation revealed that both test and control implant surfaces exhibited a monolayer of flattened SaOs-2 osteoblasts, showing complete cytoplasmic extensions and lamellopodia. While cells assumed a spindle shape, the cell bodies spanned grooves and pits of the specific micro-relief on the titanium surface. Although some adaptation to the irregularities of the underlying surface was seen, in general, no differences were noted in the morphology of the cells between test and control surfaces.

Furthermore, SEM examination failed to demonstrate any visible morphological differences between irradiated and non-irradiated control titanium surfaces. In particular, no thermal side effects, such as melting or loss of the porosity, were observed.

Representative SEM views of SaOs-2 osteoblasts after 6 days of incubation in different groups (20 kV, original magnification x 500) are presented in Figures 11a-k.

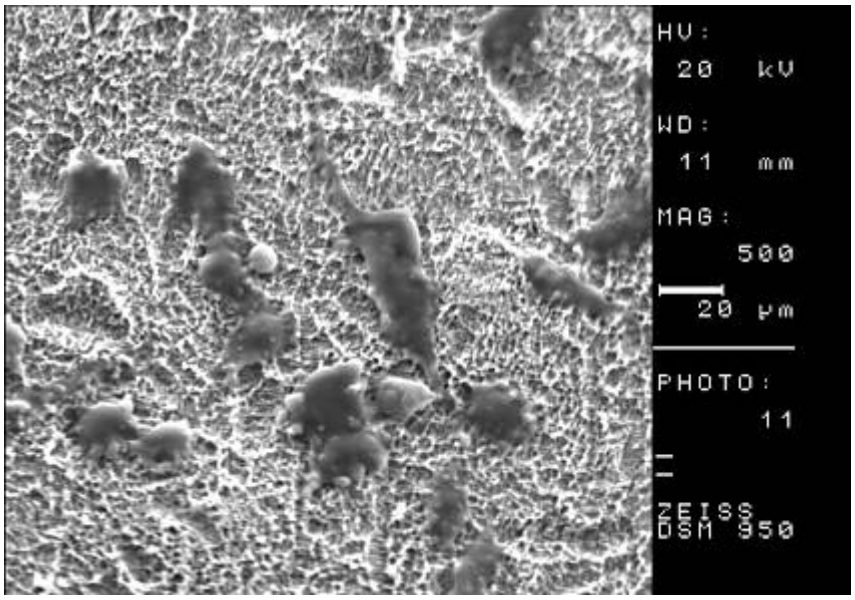
Fig. 11

a.



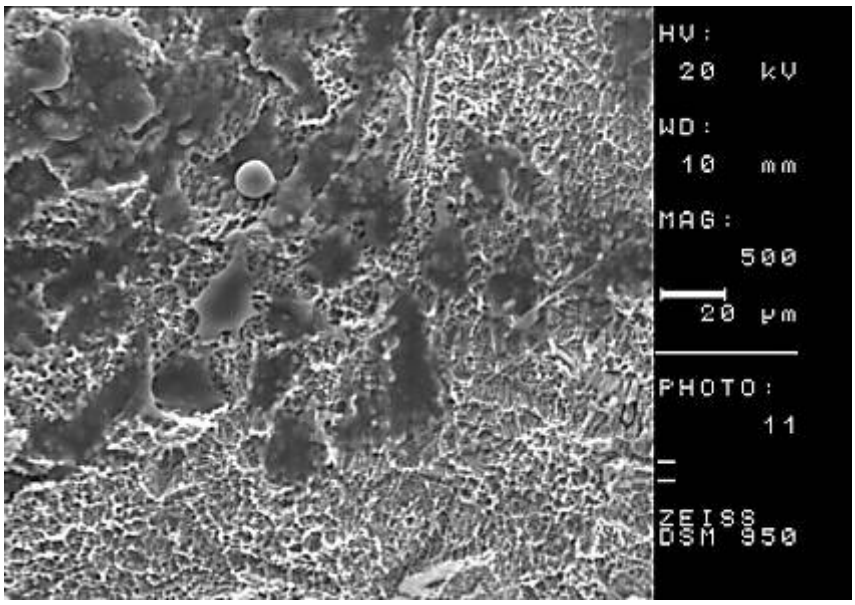
0.5 W (20 Hz)
Original magnification x 500

b.



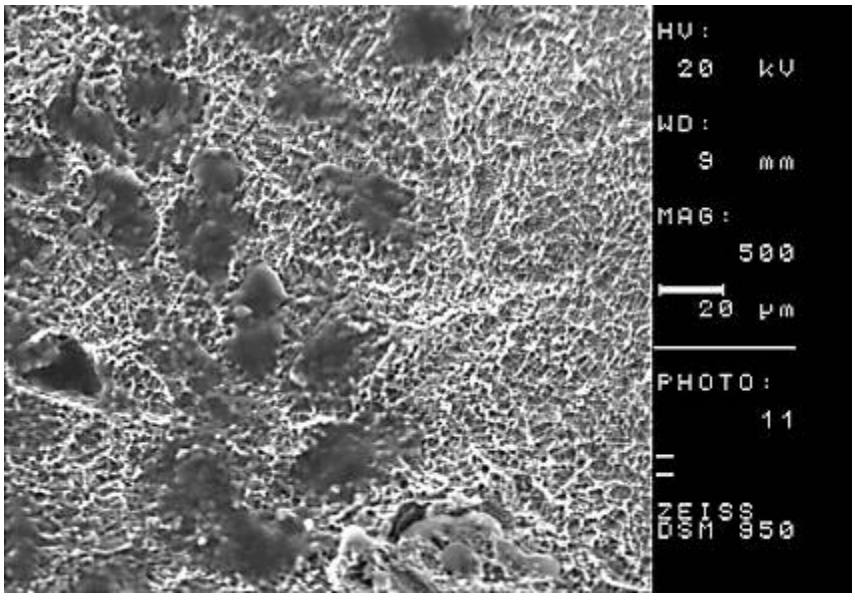
0.5 W (25 Hz)
Original magnification x 500

c.



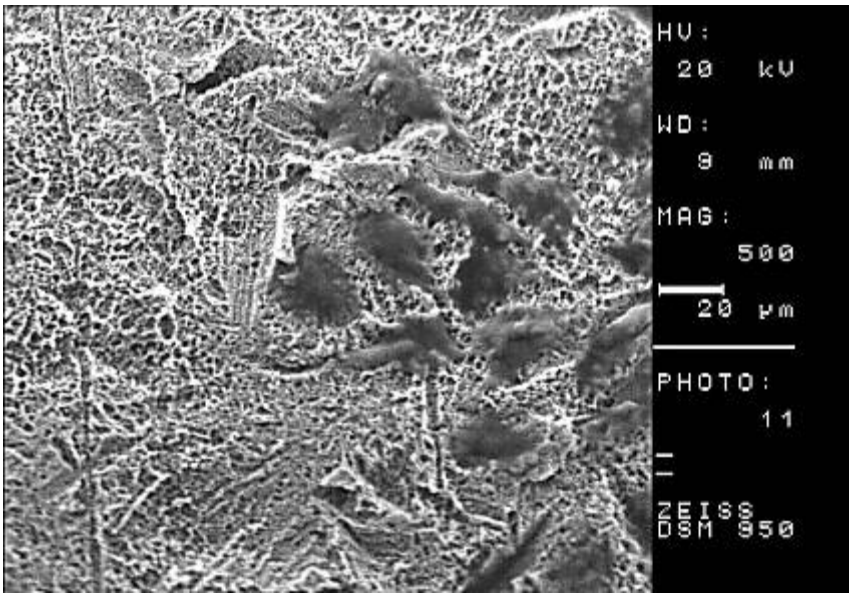
1.0 W (20 Hz)
Original magnification x 500

d.



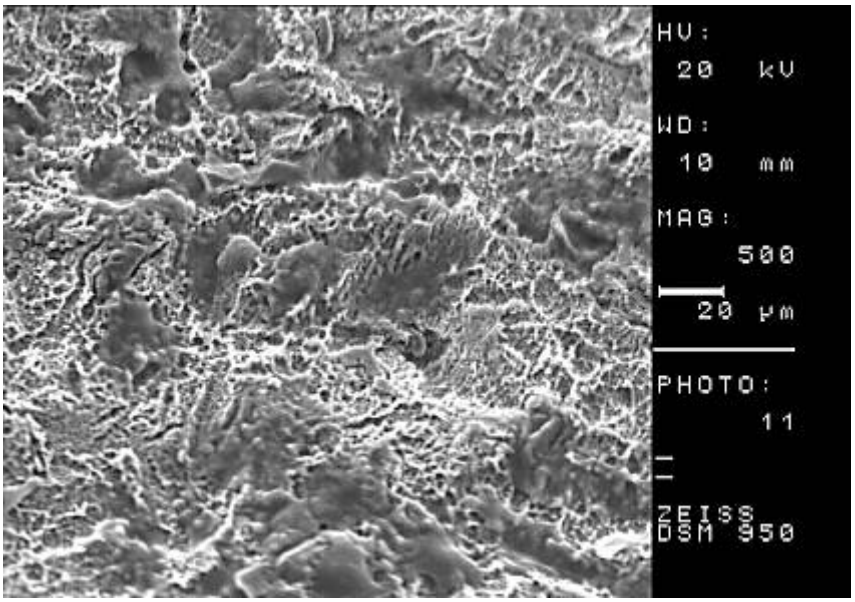
1.0 W (25 Hz)
Original magnification x 500

e.



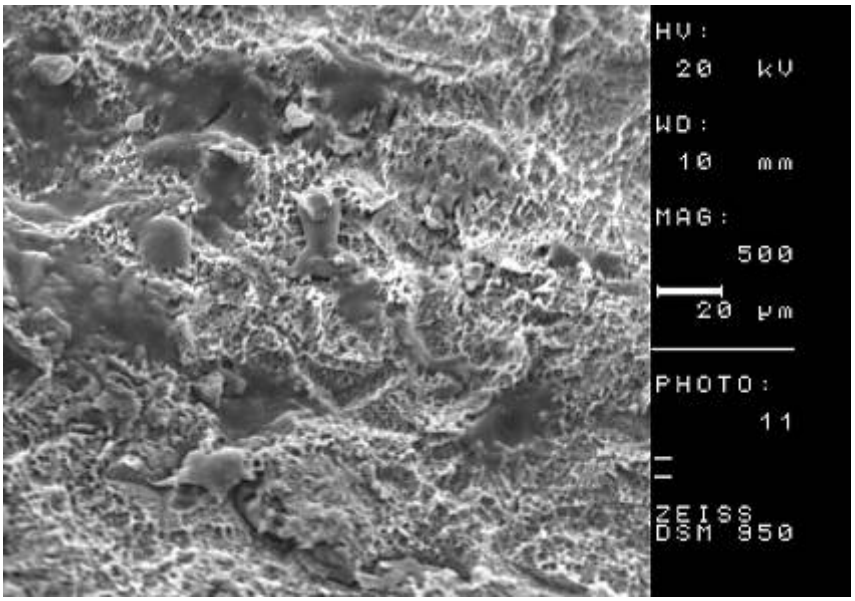
1.5 W (20 Hz)
Original magnification x 500

f.



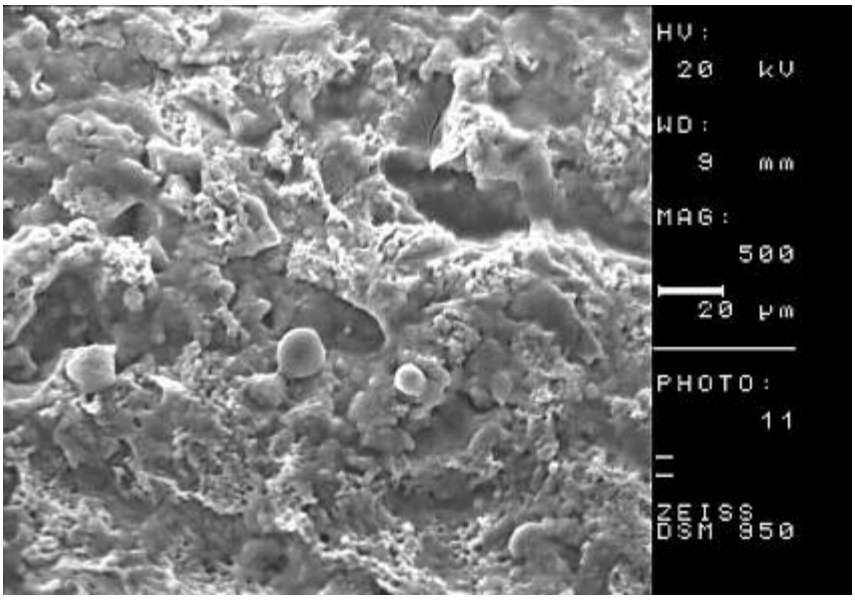
1.5 W (25 Hz)
Original magnification x 500

g.



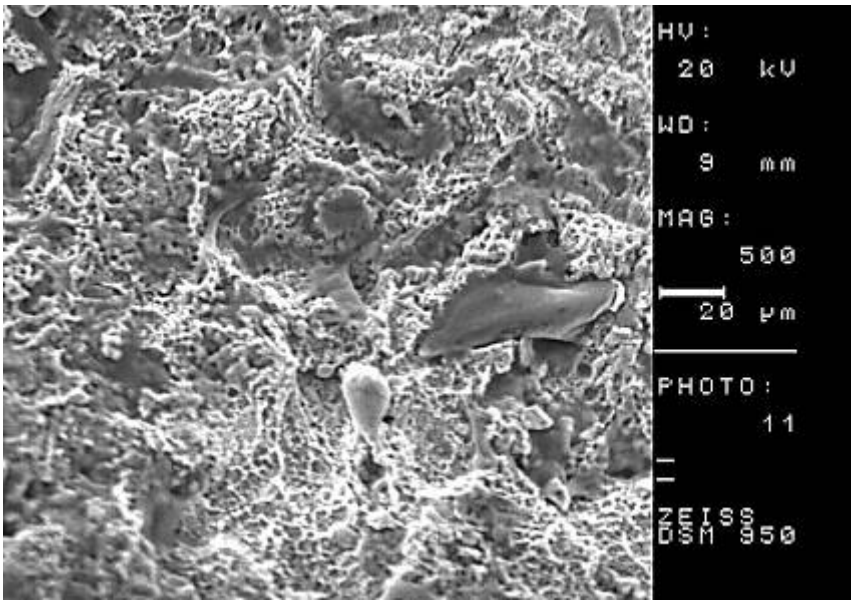
2.0 W (20 Hz)
Original magnification x 500

h.



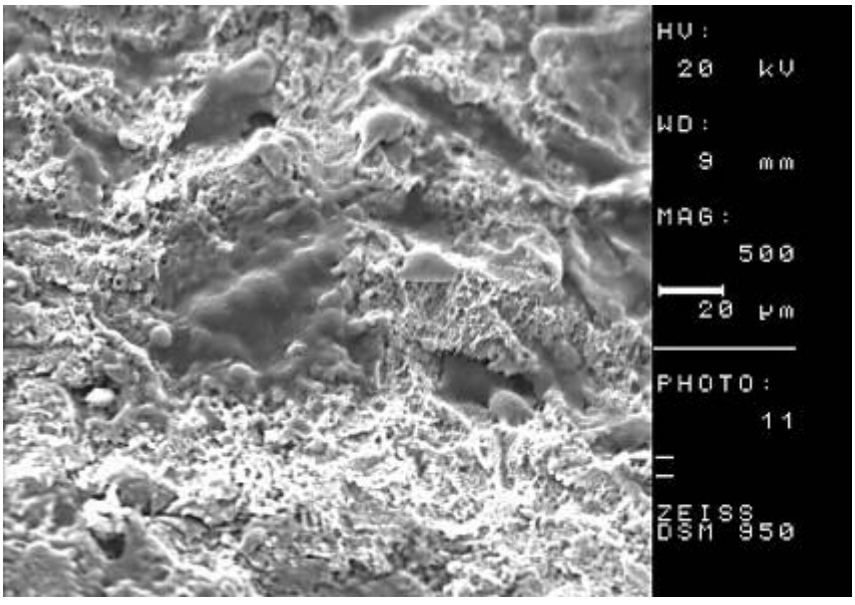
2.0 W (25 Hz)
Original magnification x 500

i.



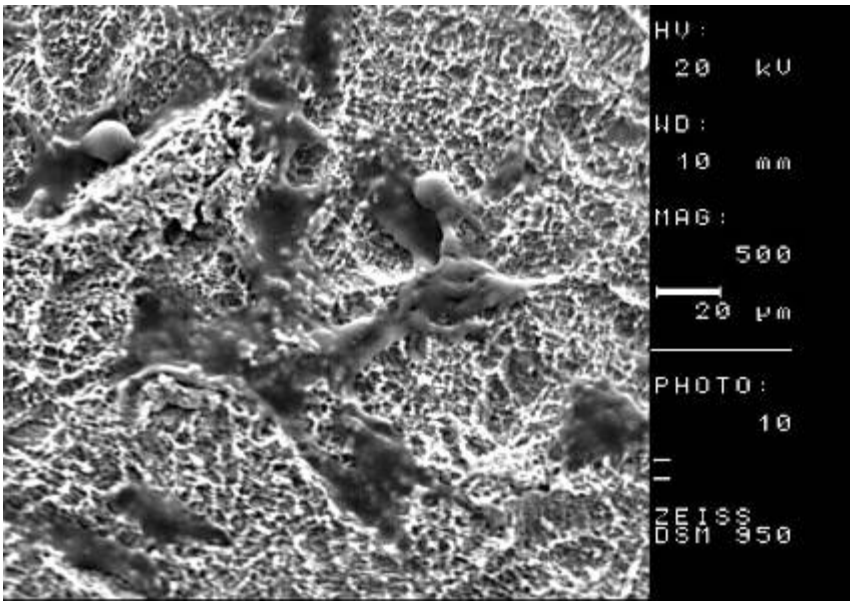
2.5 W (20 Hz)
Original magnification x 500

j.



2.5 W (25 Hz)
Original magnification x 500

k.



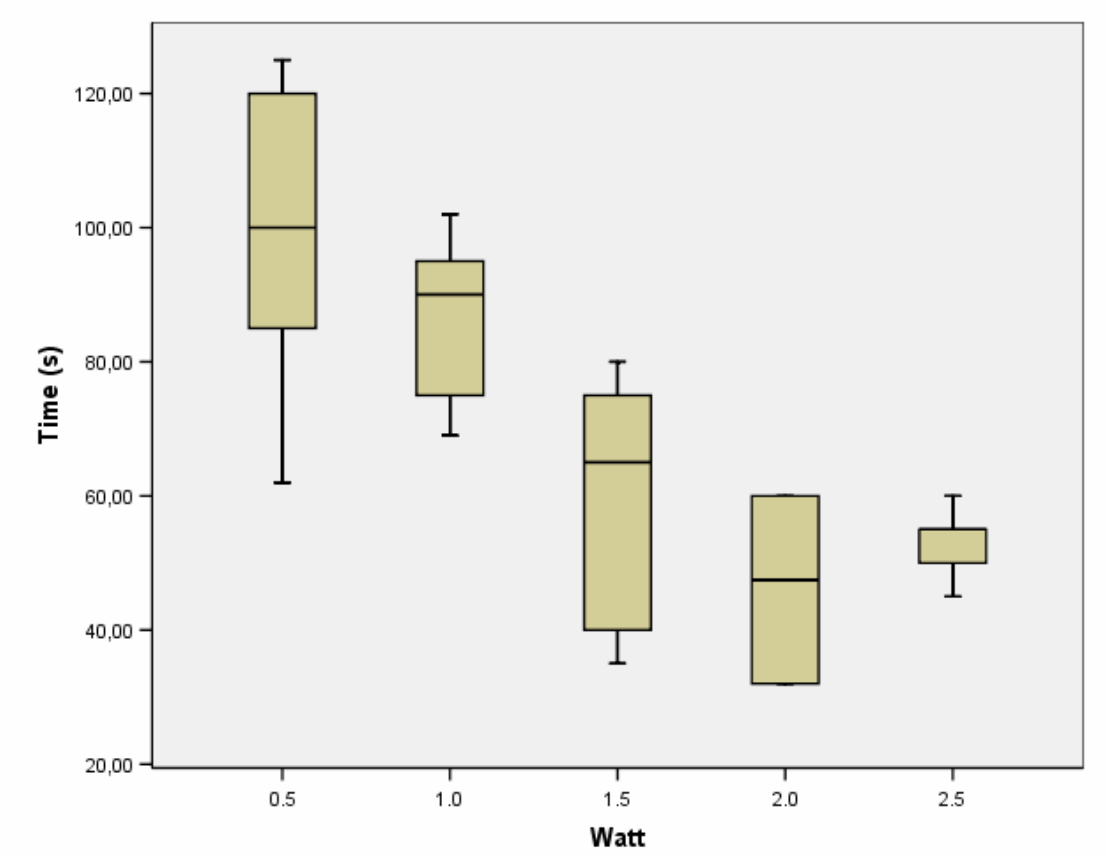
UC
Original magnification x 500

4.2. Part II

4.2.1. Residual Plaque Biofilm Areas

The time that was needed for implant surface irradiation was highest at 0.5 W (98.7±21.9), followed by 1.0 W (86.8±11.7; $P<0.01$), 1.5 W (60±17.1; $P<0.001$), 2.0 W (53.3±4.7; $P<0.05$), and 2.5 W (46.5±12.2; $P<0.01$) (Fig. 12).

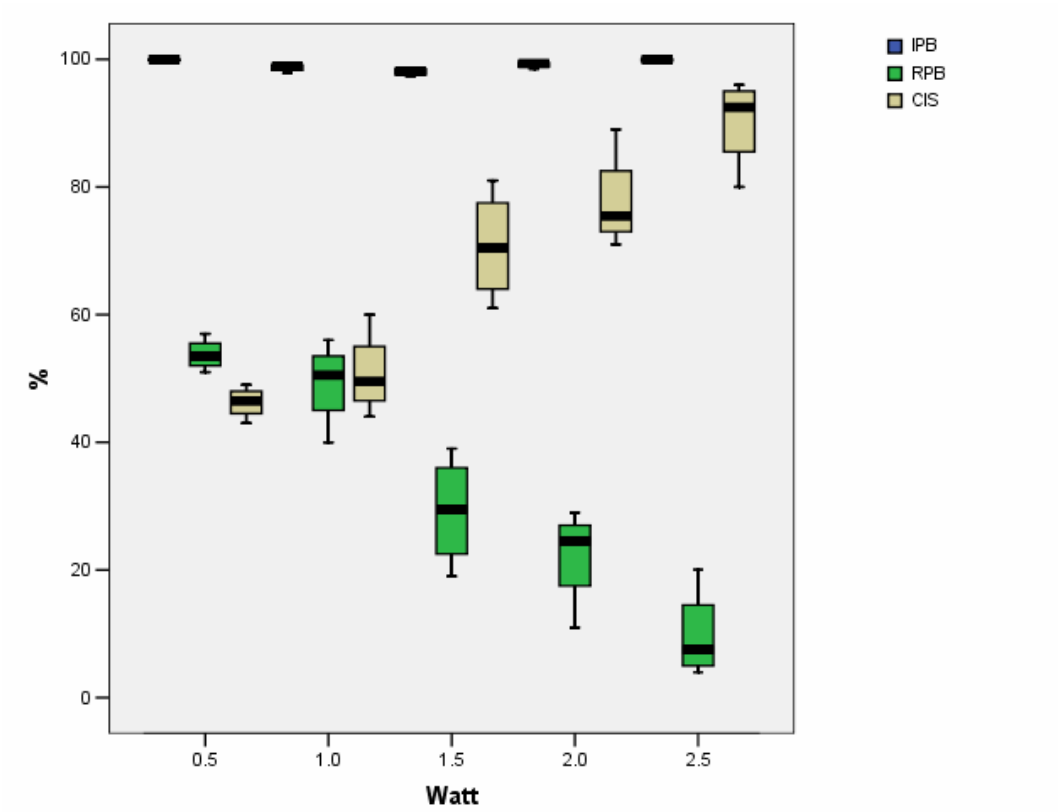
Fig. 12



Boxplots with outliers for the medians and Q1-Q3 quartiles of Treatment Time (s) in different groups.

After 24 hours, the following mean IPB areas (%) were measured: 0.5 W: 99.9±0.2; 1.0 W: 100±0.0; 1.5 W: 100±0.0; 2.0 W: 99.4±1.1; 2.5 W: 100±0.0 (P>0.05, respectively). The mean percentages of RPB and CIS areas for each energy setting are presented in Figure 13.

Fig. 13



Boxplots with outliers for the medians and Q1-Q3 quartiles of initial- (IPB), and residual plaque biofilm (RPB) areas, and clean implant surface (CIS) areas in different groups.

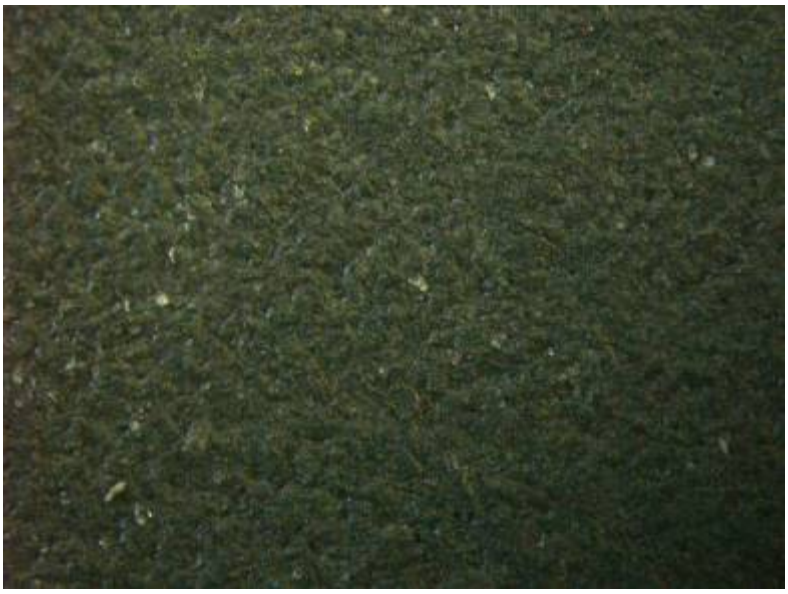
In general, all energy settings resulted in a statistically significant decrease of mean IPB areas (P<0.001, respectively). However, decreases of mean RPB areas (%) seemed to be higher with increasing energy settings (i.e. the higher the energy setting the lower the mean RPB areas). In particular, lowest RPB areas were observed following irradiation at 2.5 W

(P<0.001), followed by 2.0 W (P<0.05), and 1.5 W (P<0.001). However, the difference between 0.5 and 1.0 W was not significant (P>0.05).

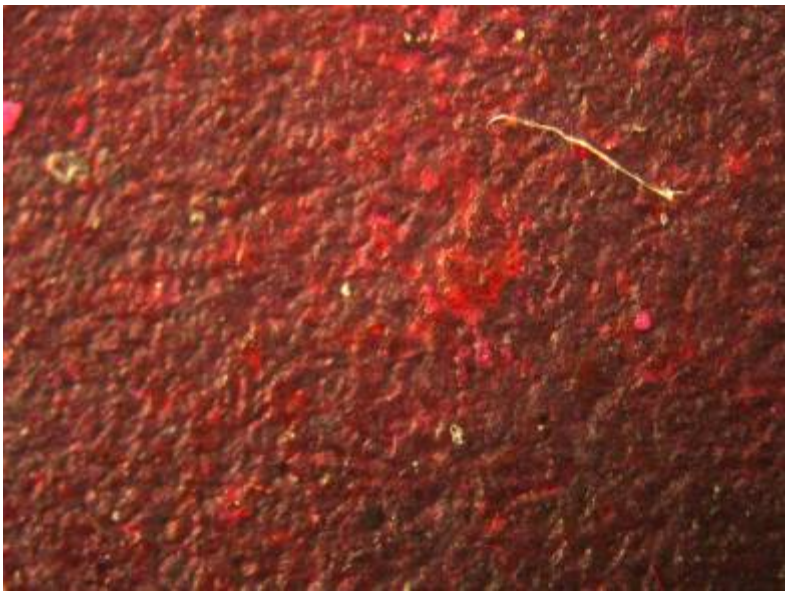
Thus, significantly highest percentages of CIS areas (%) were observed at 2.5 W (P<0.001), followed by 2.0 W (P<0.001), and 1.5 W (P<0.05). Again, the difference between 0.5 and 1.0 W was not significant (P>0.05).

Representative light microscopic views of residual plaque biofilm (RPB) areas and clean implant surface (CIS) areas (original magnification x 40) in different groups are presented in Figures 14a-l.

Fig. 14

a.  Unworn and sterile control group

b.



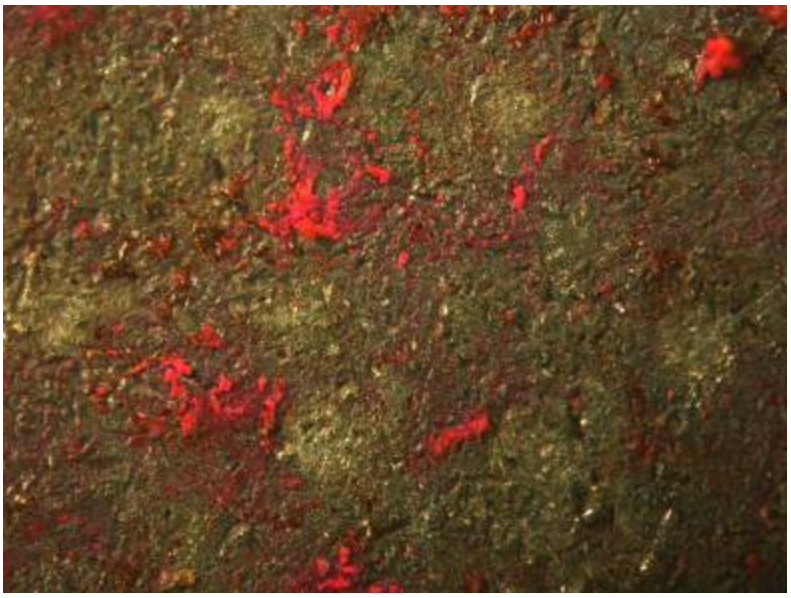
IPB: 100%

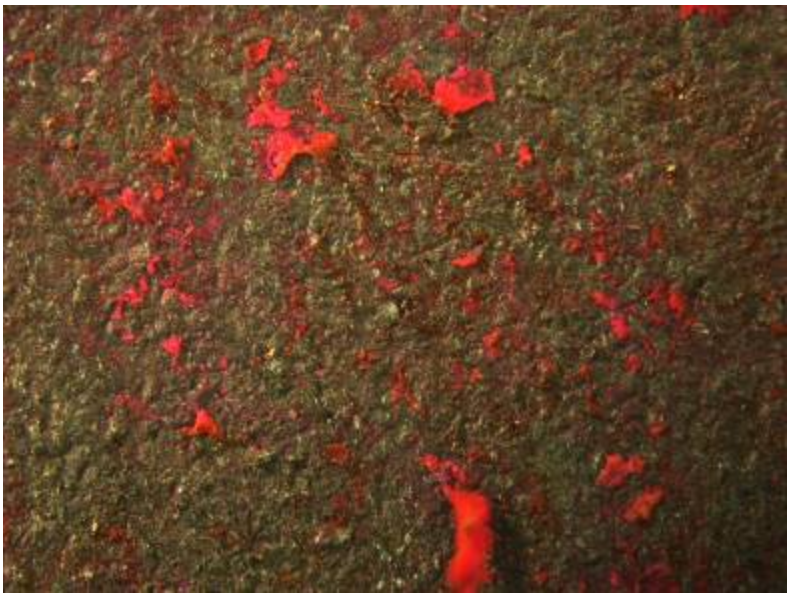
c.

0.5 W (25 Hz)

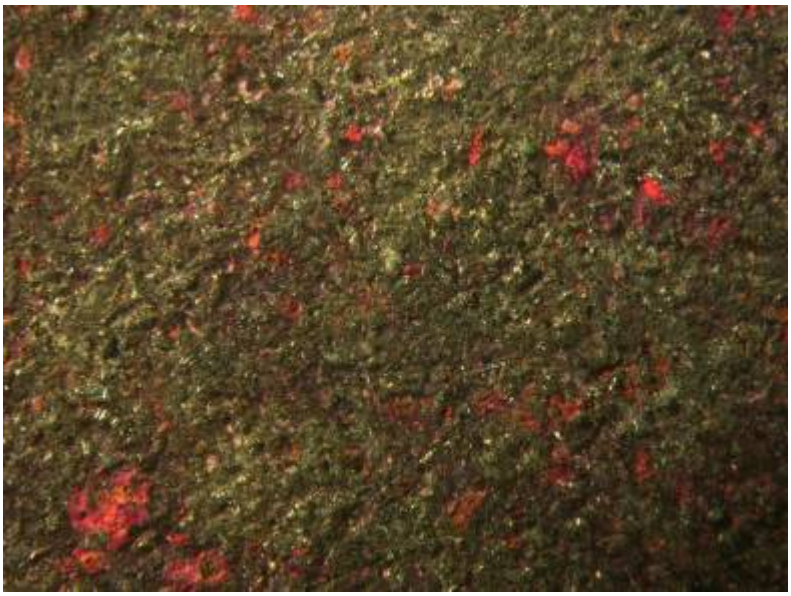
d.

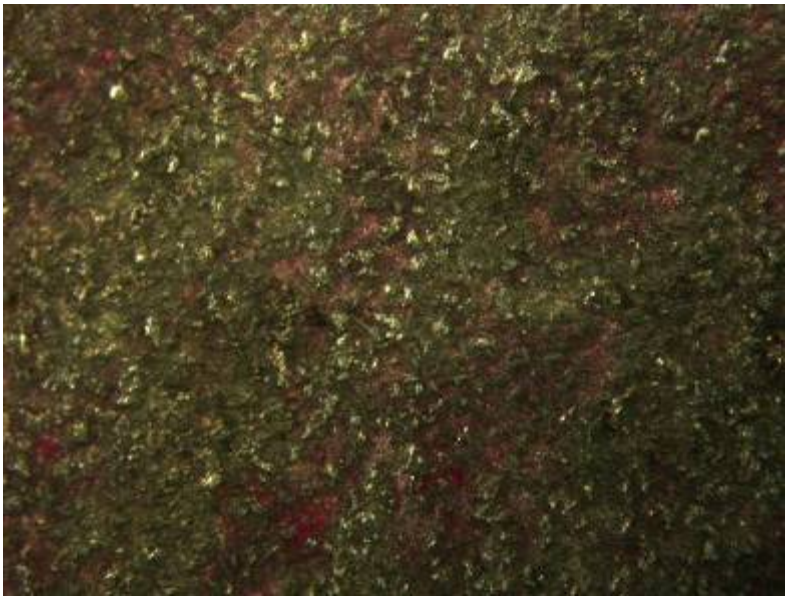
0.5 W (25 Hz)

e.  1.0 W (25 Hz)

f.  1.0 W (25 Hz)

g.  1.5 W (25 Hz)

h.  1.5 W (25 Hz)

i.  2.0 W (25 Hz)

j.  2.0 W (25 Hz)

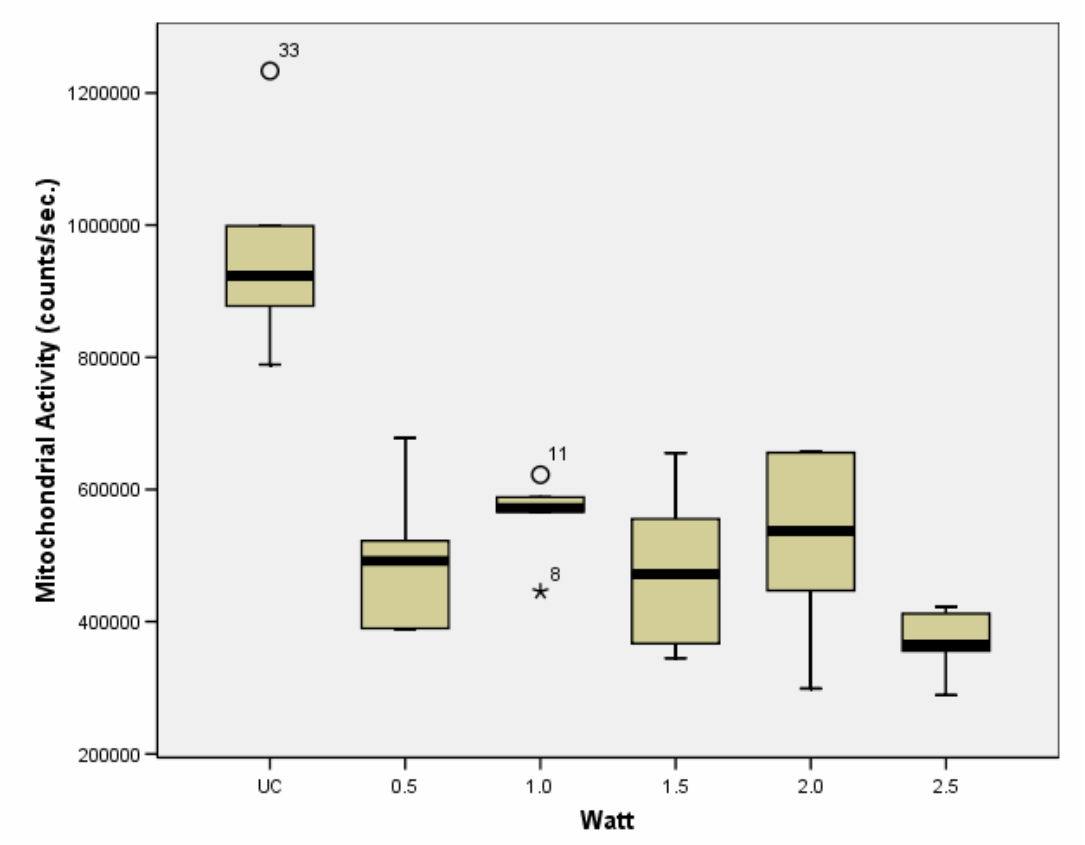
k.  2.5 W (25 Hz)

l.  2.5 W (25 Hz)

4.2.2. Cell viability

During the experimental period, there were no signs of any bacterial or fungal contamination of the well chambers. MA of SaOs-2 osteoblasts, expressed as luminescent output (counts per second), is presented in Figure 14. In comparison to the UC group, measurement of luminescent output revealed significantly lower values following irradiation using ERCL either at 0.5, 1.0, 1.5, and 2.0 W (P<0.001; respectively). However, lowest mean values for MA were observed following irradiation at 2.5 W, even reaching statistical significance in comparison to 0.5, 1.0, 1.5, and 2.0 W (P<0.001; respectively) (Fig. 14).

Fig. 14



Boxplots with outliers for the medians and Q1-Q3 quartiles of mitochondrial activity of SaOs-2 osteoblasts (counts per second) at plaque contaminated implant surfaces (Part II) in different groups.

5. Discussion

The results of the present study have indicated that ERCL, used with a cone-shaped fibre tip in contact mode, did not cause any thermal damages on SLA titanium implant surfaces at energy settings of up to 2.5 W. Since SEM observation revealed no identifiable differences between 20 Hz and 25 Hz regarding cell morphology and surface changes, the latter pulse repetition rate was chosen in order to optimize the efficiency of plaque biofilm removal. Indeed, it was observed that in an energy and time dependent manner, this kind of laser resulted in a statistically significant decrease of IPB areas grown on SLA titanium implants.

In this context, it has to be pointed out that the design of the intraoral splint to collect an *in vivo* biofilm followed the pattern described in previous studies (Auschill et al., 2001; Hahn et al., 1992; Schwarz et al., 2005b; Schwarz et al., 2006a). In particular, the inserted titanium discs were turned towards the palate in order to allow a better and more comfortable plaque growth without any tongue disturbance, but with some space to provide a nutritious aqueous environment.

The observation that SLA implant surfaces facilitate early plaque biofilm formation is in agreement with previous observations which have shown that rough surfaces accumulate a homogenous and mature plaque biofilm within 24 h (Rimondini et al., 1997; Schwarz et al., 2005b; Schwarz et al., 2006a; Siegrist et al., 1991). Moreover, a previous study investigated the maturation of supragingival microbial plaque on smooth titanium and that of subgingival plaque on plasma-sprayed titanium. In particular, it was observed that the sequence of appearance of various microbial morphotypes in supra- and subgingival plaque was comparable regardless of surface (Gatewood et al., 1993). Similar results were also reported by Leonhardt et al. (Leonhardt et al., 1995), since a comparable qualitative and quantitative bacterial colonization was observed on titanium, hydroxyapatite and amalgam surfaces over

an observation period of 72 hours. On the other hand, however, several studies have reported on a close relationship between surface roughness and the amount of plaque accumulation (Bollen et al., 1997; Quirynen et al., 1993; Rimondini et al., 1997; Zitzmann et al., 2002), even though microtopographie apparently had no effect on species selection (Siegrist et al., 1991). Consequently, titanium surfaces exhibiting Ra values of < 0.088 µm were recommended for transgingival and healing implant components (Rimondini et al., 1997). Moreover, Quirynen et al. (1989, 1990) reported that surface roughness had a greater influence on plaque accumulation and composition than did the surface free energy, even though hydrophobic surfaces (teflon) harboured 10 x less plaque than hydrophilic ones (enamel).

To the best of our knowledge, these are the first data reporting on the effectiveness of ERCL, used with a cone-shaped fibre tip, for the removal of plaque biofilms grown on structured titanium implants. Due to its radial and axial pattern of radiation, this specific fibre may also allow for an irradiation of screw-typed implants. However, this issue has to be investigated in further experimental studies. The present results corroborate, to a certain extent, previous findings from an experimental *in vitro* study (Miller, 2004). In this investigation, ERCL (600µm fibre, 6.0 W, 32% water and 100% air) was compared with citric acid treatment of titanium plasma sprayed and hydroxyapatite coated implant surfaces. It was concluded that laser ablation using ERCL is highly efficient at removing potential contaminants on roughened implant surfaces while demonstrating no thermal side effects (Miller, 2004). So far, the removal of IPB areas from SLA titanium implant surfaces has been reported for plastic curettes supported by irrigation with a chlorhexidine digluconate solution (PC+CHX), various ultrasonic devices (US) and an Er: YAG laser (Schwarz et al., 2005b; Schwarz et al., 2006a). While PC+CHX revealed mean RPB areas of 58.5±4.9 to 61.1±11.4%, US exhibited significantly lower mean RPB areas, ranging from 36.8±4.5 to 28.3±2.0% (Schwarz et al.,

2005b; Schwarz et al., 2006a). In contrast, significantly lowest RPB areas were observed following irradiation using an Er: YAG laser (5.8±5.1%) (Schwarz et al., 2005b).

Recently, a cone-shaped quartz glass fibre tip (diameter: cylinder 1mm; conical tip 0.5 mm), emitting an axial and radial laser beam, has also been designed for nonsurgical instrumentation of peri-implant pockets using an Er: YAG laser. The specific pattern of radiation was generated in order to enable even irradiation of screw-typed dental implants. However, the axial and radial components of radiation may also unintentionally damage the adjacent alveolar bone during laser instrumentation. Indeed, the results of a recent cell culture study have shown that mitochondrial activity of SaOs-2 osteoblasts was significantly reduced after Er:YAG laser irradiation using a cone-shaped glass fibre tip at energy settings of 40, 60, 80 and 100 mJ at 10 Hz (energy densities of 5.08, 7.62, 10.16 and 12.7 Jcm^2) (Schwarz et al., 2004). Each energy setting was used at a distance of 1, 2 and 3 mm between the application tip and the bottom of the culture plate. Time of exposure was constantly set at 10 seconds. Non-irradiated cell cultures served as positive controls (n=10). Following irradiation, mitochondrial activity of the cells was measured using a luminescent cell viability assay. After laser irradiation, MA of SaOs-2 osteoblasts was significantly reduced when compared to non-irradiated cells, irrespective of the used energy setting or distance between the application tip and the bottom of the culture plate. However, mitochondrial activity increased significantly with decreasing energy settings and increasing distances.

Based on these results, detrimental effects of laser irradiation may be reduced by decreasing energy settings on the one hand and increasing the distance between the application tip and alveolar bone on the other hand. However, during nonsurgical instrumentation it may be difficult to estimate depth and width of the intrabony component around dental implants. On

the other hand it is important to realize that results obtained by using an *in vitro* experimental model can not recreate the complex interactions of cells *in vivo*.

Moreover, recent results from a controlled experimental study have demonstrated that an Er:YAG laser, used with this kind of fibre tip, does not damage titanium surfaces and subsequently does not influence the attachment rate of SaOs-2 osteoblasts *in vitro* (Schwarz et al., 2003b). A total of 168 titanium discs with 4 different types of surfaces (sand-blasted and acid etched-SLA, titanium plasma sprayed-TPS, machine polished-MP, and hydroxyapatite coated-HA) were used to evaluate cell attachment. The samples have been equally and randomly assigned to the following groups: (1) Er:YAG laser at an energy level of 100 mJ/pulse and 10 Hz (12.7 J/cm^2) using a cone-shaped fibre tip, or (2) Vector® ultrasonic system using carbon fibre tips, or (3) untreated control. The discs were placed into culture plates, covered with a solution of SaOs-2 cells, and incubated for 7 days. The specimens were then washed with phosphate buffer to remove cells not attached to the surface, and the adherent cells were stained with hematoxiline-eosine. Cells were counted using a reflected light microscope and the cell density per mm^2 was calculated. Additionally, cell morphology and surface alterations of the titanium discs after treatment were investigated using SEM.

All titanium discs treated with the Er: YAG laser demonstrated a comparable cell density per mm^2 as the untreated control surfaces. There was a significant decrease in the number of cells that attached to the implant surfaces treated with the ultrasonic system. The SEM examination showed no visible differences between lased and control titanium surfaces. All surfaces treated with the ultrasonic system showed conspicuous surface damages and debris of the used carbon fibres (Schwarz et al., 2003b).

So far, there are only few data available describing the effects of an Er:YAG laser on the surface characteristics of differently coated titanium discs (Kreisler et al., 2002c; Rechmann et al., 2000). In a recent study, Kreisler et al. (2002a) observed conspicuous surface damages, such as melting and glazing, at energy densities of 8.9 Jcm^2 in TPS surfaces, 11.2 Jcm^2 in SLA surfaces, 17.8 Jcm^2 in hydroxyapatite (HA)-coated surfaces and 28 Jcm^2 in polished titanium surfaces. However, the laser beam was used in non-contact mode without water cooling and the angle of irradiation was perpendicular to the surface (90°). In a comparable study, first micro-morphological changes in SLA and TPS titanium surfaces occurred at an energy density of 7 Jcm^2 (Rechmann et al., 2000). This discrepancy might be explained by the fact that the fibre tip was guided parallel to the titanium surfaces in contact mode under sufficient water cooling (Schwarz et al., 2003b). In this context, it is important to point to the results of a previous study which have shown that the angulation of the application tip has a strong influence on the amount of root substance removal using Er:YAG laser radiation for periodontal treatment (Folwaczny et al., 2001). Furthermore, it should be pointed out that permanent water cooling might cause less damage than irradiation without water irrigation.

The results of a recent *in vitro* investigation have pointed to a high bactericidal potential of the Er:YAG laser on rough titanium surfaces (Kreisler et al., 2002d). Commercially available SLA, TPS and HA coated titanium discs were incubated with a suspension of *Streptococcus sanguis* and irradiated at energy settings of 60 and 120 mJ at 10 Hz (7.62-15.24 J/cm^2) for 60 seconds without water irrigation in non-contact mode. Non-irradiated control titanium discs revealed the following bacterial counts: (log): SLA: 6.38×10^4; TPS: 6.25×10^5; HA: 2.73×10^5. Subsequent to irradiation, bacterial counts were significantly reduced (log) to: SLA: 3.13×10^2; TPS: 2.50×10^3; HA: 4.38×10^3 at a pulse energy of 60 mJ (7.62 J/cm^2), and to: SLA: 5.00×10^1; TPS: 3.88×10^2; HA: 4.12×10^2 at a pulse energy of 120 mJ (15.24 J/cm^2). However, a complete bacterial reduction following laser irradiation could not be observed,

irrespective of energy setting. However, at these laser parameters, no excessive temperature elevations (<47ºC), which might have influenced bacterial reduction additionally, or morphological implant surface alterations were detected (Kreisler et al., 2002d). Similar results were also observed by Kreisler et al. (2005). Titanium platelets with a SLA surface were coated with bovine serum albumin and incubated with a suspension of *Porphyromonas gingivalis*. Contaminated specimens were randomly irradiated with an Er: YAG Laser (60 mJ/Pulse, 10 Hz; 7.62 J/cm^2), or treated with an air powder system. After the respective treatment, human gingival fibroblasts were incubated on the specimens. Cell proliferation was significantly reduced on contaminated and non-treated specimens when compared to sterile specimens. In both treatment groups, cell proliferation was not significantly different from that on sterile control specimens. However, the air powder system led to microscopically visible alterations of the implant surface whereas laser-treated surfaces remained unchanged (Kreisler et al., 2005).

Previous findings from a case report study evaluated the effectiveness of an Er:YAG Laser for the removal of subgingival debris from titanium implants under clinical conditions (Schwarz et al., 2003a). This investigation was conducted on eight implants (SLA and TPS) of two patients, considered for explantation due to severe bone loss and inflammation. Immediately before explantation, six implants were instrumented subgingivally with the Er: YAG laser (12.7 J/cm^2), while two implants served as a control. All titanium implants were examined using scanning electron microscopy by one calibrated and blinded examiner. In comparison to the untreated control group, nonsurgical instrumentation of titanium implants with an Er: YAG laser resulted in an effective removal of subgingival calculus without leading to any thermal damages. However, all samples of the test group revealed amounts of residual debris which should be taken into account under clinical conditions (Schwarz et al., 2003a). Most recently, the Er:YAG laser at 30 mJ/pulse and 30 Hz with water spray has also been reported

to be capable of effectively removing plaque and calculus from implant abutments without injuring their surfaces (Matsuyama et al., 2003).

Most recently, results of a pilot study have also indicated that nonsurgical treatment of peri-implantitis with an Er:YAG laser may lead to significant clinical improvements (Schwarz et al., 2005a). Twenty patients with moderate to advanced peri-implantitis lesions were randomly treated with either (1) an Er:YAG laser using a cone-shaped glass fiber tip at an energy setting of 100 mJ/pulse and 10 Hz (12.7 J/cm^2), or (2) mechanical debridement using plastic curettes and antiseptic therapy with chlorhexidine digluconate (0.2 %) as control group.(C). The following clinical parameters were measured at baseline, 3 and 6 months after treatment by one blinded and calibrated examiner: Plaque index (PI), bleeding on probing (BOP), probing depth (PD), gingival recession (GR) and clinical attachment level (CAL). In both groups, there were no signs of any adverse effects that could be associated with the specific treatment procedure.

However, in the control group, one patient with two implants was discontinued from the study due to persisting pus formation eight weeks after treatment. Mean values of BOP decreased in the laser group from 83% at baseline to 31% after 6 months and in the control group from 80% at baseline to 58% after 6 months. The difference between both groups was statistically significant. The laser treated sites demonstrated a mean CAL change from 5.8±1.0 mm at baseline to 5.1±1.1 mm after 6 months. The C sites demonstrated a mean CAL change from 6.2±1.5 mm at baseline to 5.6±1.6 mm after 6 months.

After 6 months, the difference between both groups was statistically non significant (Schwarz et al., 2005a). In this context, it must be pointed out, that these clinical results were only based on a short-term observation of 6 months and a small study population.

All these data, taken together with the results of the present study seem to indicate that, in an energy dependent manner, the effectiveness of ERCL for plaque biofilm removal seems to be superior to that of PC+CHX and US, and within the range of that reported for an Er:YAG laser.

However, the latter situation was merely observed at an energy setting of 2.5 W (25 Hz), since mean RPB area was 9.8±6.2. From a clinical point of view, however, it is important to realize that a supragingival plaque biofilm, collected artificially after a period of 24 hours is non-mineralized, whereas subgingival calculus is defined as mineralized plaque that is permeated with crystals of various calcium phosphates (Schroeder, 1965). Furthermore, it has been reported that subgingivally, rough titanium surfaces harboured 25 times more bacteria, with increased proportions of Gram-negative anaerobic and facultative anaerobic species (Alcoforado et al., 1991; Quirynen et al., 1993; Rams et al., 1984). Therefore, the removal of subgingival plaque biofilms from rough titanium implants may be more difficult to achieve than the removal of non-mineralized supragingival plaque biofilms.

When interpreting the present results, it must also be emphasized that in all treatment groups MA of SaOs-2 osteoblasts was statistically significant lower compared to non-contaminated and untreated control titanium discs. Furthermore, it was observed that with increasing energy settings, the present results revealed an inverse relation between RPB areas and MA. This was particularly true for 2.5 W (25 Hz), since this specific energy setting exhibited significantly lowest MA of SaOs-2 osteoblasts grown on previously contaminated implant surfaces. Even though MA also tended to be lowest at non-contaminated implant surfaces using 2.5W at both 20 Hz and 25 Hz, these differences did not reach statistical significance. There might be several explanations for this discrepancy. First of all, it has to be noted that even though SEM examination failed to demonstrate any thermal damages of SLA titanium surfaces at 2.5 W

(25 Hz), it is impossible to estimate to what extent this energy setting might have influenced the chemical composition of the dioxide layer and subsequently MA of cells. In this context, it must be emphasized that MA was measured using an ATP-based luminescent cell viability assay, which has been reported to be more sensitive than other methods (Ahmad et al., 1999; Cree et al., 1995; Maehara et al., 1987; Petty et al., 1995). The luminescent signal, generated during cell lysis, is proportional to the amount of ATP present. Furthermore, the amount of ATP has been shown to be directly proportional to the number of viable cells present in culture (Crouch et al., 1993).

In the present study, we used human osteosarcoma-derived SAOS-2 cells that have been well characterized as osteoblast-like cells (Murray et al., 1987; Rodan et al., 1987). However, transformed cell lines have their own limitations as some of the cell characteristics are different from those of primary cells. Nevertheless, in long-term *in vitro* mineralization studies, normal human osteoblast cultures responded in a similar way to implant surfaces such as SAOS-2 cells, but with approximately two-third less calcification (Ahmad et al., 1999).

Another possible explanation for the reduced MA in all treatment groups may be due to RPB areas which might have influenced viability of SaOs-2 cells. Indeed, recent studies have demonstrated that plaque biofilms may alter the surface characteristics of titanium surfaces (Mouhyi et al., 2000). It was presumed that bacterial contamination of a titanium surface may affect its dioxide layer resulting in a lower surface energy and subsequently reduced tissue integration (Baier and Meyer, 1988; Sennerby and Lekholm, 1993). These observations might also be supported by the results of a recent experimental study, which have shown that even though ERL facilitated the removal of IPB areas without causing any thermal damages to the implant surfaces, it also failed to re-establish the biocompatibility of previously contaminated titanium surfaces (Schwarz et al., 2005b). Indeed, in comparison to non-contaminated and

untreated control specimens, MA was statistically significant lower in the ERL group (Schwarz et al., 2005b). However, similar results were also observed for PC+CHX and US (Schwarz et al., 2005b; Schwarz et al., 2006a).

Finally, it must also be pointed out, that currently there is still a lack of clinical data evaluating the subgingival microflora associated with peri-implant infections following ERCL irradiation *in vivo*. Therefore, further studies are needed in order to compare the effectiveness of this treatment modality on microbiological changes to that of adjunctive local or systemic antibiotic therapy. Another point of interest may be the evaluation of the relative cost-effectiveness of different treatment approaches. From a clinical point of view, it should also be taken into account that a huge number of different implant types and surface characteristics complicate a generalization of the present results.

Within the limits of the present study, it was concluded that even though ERCL exhibited a high efficiency to remove IPB areas in an energy dependent manner, it failed to re-establish the biocompatibility of contaminated titanium surfaces.

6. References

Abrahamsson I, Berglundh T, Wennstrom J, Lindhe J (1996). The peri-implant hard and soft tissues at different implant systems. A comparative study in the dog. *Clin Oral Implants Res* 7(3):212-9.

Abrahamsson I, Berglundh T, Glantz PO, Lindhe J (1998). The mucosal attachment at different abutments. An experimental study in dogs. *J Clin Periodontol* 25(9):721-7.

Abrahamsson I, Zitzmann NU, Berglundh T, Linder E, Wennerberg A, Lindhe J (2002). The mucosal attachment to titanium implants with different surface characteristics: an experimental study in dogs. *J Clin Periodontol* 29(5):448-55.

Ahmad M, McCarthy MB, Gronowicz G (1999). An *in vitro* model for mineralization of human osteoblast-like cells on implant materials. *Biomaterials* 20(3):211-20.

Albrektsson T (1983). Direct bone anchorage of dental implants. *J Prosthet Dent* 50(2):255-61.

Albrektsson T, Zarb G, Worthington P, Eriksson AR (1986). The long-term efficacy of currently used dental implants: a review and proposed criteria of success. *Int J Oral Maxillofac Implants* 1(1):11-25.

Albrektsson T, Isidor F (1994). Consensus report of session IV. In Proceedings of the First European Workshop on Periodontology, eds. Lang, N.P. & Karring, T. *pp. 365-369. London: Quintessence.*

Alcoforado GA, Rams TE, Feik D, Slots J (1991). Microbial aspects of failing osseointegrated dental implants in humans. *J Parodontol* 10(1):11-8.

Ando Y, Aoki A, Watanabe H, Ishikawa I (1996). Bactericidal effect of erbium YAG laser on periodontopathic bacteria. *Lasers Surg Med* 19(2):190-200.

Aoki A, Ando Y, Watanabe H, Ishikawa I (1994). *In vitro* studies on laser scaling of subgingival calculus with an erbium: YAG laser. *J Periodontol* 65(12):1097-106.

Augthun M, Tinschert J, Huber A (1998). *In vitro* studies on the effect of cleaning methods on different implant surfaces. *J Periodontol* 69(8):857-64.

Aukhil I, Schaberg TV, Greco GW, Simpson DM (1988). Surgical versus non-surgical treatment and recurrent periodontal disease in beagle dogs. *J Clin Periodontol* 15(2):99-105.

Auschill TM, Arweiler NB, Netuschil L, Brecx M, Reich E, Sculean A, et al. (2001). Spatial distribution of vital and dead microorganisms in dental biofilms. *Arch Oral Biol* 46(5):471-6.

Bach G, Neckel C, Mall C, Krekeler G (2000). Conventional versus laser-assisted therapy of periimplantitis: a five-year comparative study. *Implant Dent* 9(3):247-51.

Baier RE, Meyer AE (1988). Implant surface preparation. *Int J Oral Maxillofac Implants* 3(1):9-20.

Baron M, Haas R, Dortbudak O, Watzek G (2000). Experimentally induced peri-implantitis: a review of different treatment methods described in the literature. *Int J Oral Maxillofac Implants* 15(4):533-44.

Barone A, Covani U, Crespi R, Romanos GE (2002). Root surface morphological changes after focused versus defocused CO2 laser irradiation: a scanning electron microscopy analysis. *J Periodontol* 73(4):370-3.

Becker W, Becker BE, Newman MG, Nyman S (1990). Clinical and microbiologic findings that may contribute to dental implant failure. *Int J Oral Maxillofac Implants* 5(1):31-8.

Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljenberg B, Thomsen P (1991). The soft tissue barrier at implants and teeth. *Clin Oral Implants Res* 2(2):81-90.

Berglundh T, Lindhe J, Marinello C, Ericsson I, Liljenberg B (1992). Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. *Clin Oral Implants Res* 3(1):1-8.

Berglundh T, Lindhe J, Jonsson K, Ericsson I (1994). The topography of the vascular systems in the periodontal and peri-implant tissues in the dog. *J Clin Periodontol* 21(3):189-93.

Berglundh T, Lindhe J (1996). Dimension of the periimplant mucosa. Biological width revisited. *J Clin Periodontol* 23(10):971-3.

Blomlöf J, Lindskog S (1995). Root surface texture and early cell and tissue colonization after different etching modalities. *Eur J Oral Sci* 103(1):17-24.

Blomlöf JP, Blomlöf LB, Lindskog SF (1997). Smear layer formed by different root planing modalities and its removal by an ethylenediaminetetraacetic acid gel preparation. *Int J Periodontics Restorative Dent* 17(3):242-9.

Blomlöf JP, Blomlöf LB, Lindskog SF (1996). Smear removal and collagen exposure after non-surgical root planing followed by etching with an EDTA gel preparation. *J Periodontol* 67(9):841-5.

Bollen CM, Papaioanno W, Van Eldere J, Schepers E, Quirynen M, van Steenberghe D (1996). The influence of abutment surface roughness on plaque accumulation and peri-implant mucositis. *Clin Oral Implants Res* 7(3):201-11.

Bollen CM, Lambrechts P, Quirynen M (1997). Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: a review of the literature. *Dent Mater* 13(4):258-69.

Bowers GM, Chadroff B, Carnevale R, Mellonig J, Corio R, Emerson J, et al. (1989). Histologic evaluation of new attachment apparatus formation in humans. Part III. *J Periodontol* 60(12):683-93.

Brägger U, Hugel-Pisoni C, Burgin W, Buser D, Lang NP (1996). Correlations between radiographic, clinical and mobility parameters after loading of oral implants with fixed partial dentures. A 2-year longitudinal study. *Clin Oral Implants Res* 7(3):230-9.

Bunyaratavej P, Wang HL (2001). Collagen membranes: a review. *J Periodontol* 72(2):215-29.

Buser D, Weber HP, Lang NP (1990). Tissue integration of non-submerged implants. 1-year results of a prospective study with 100 ITI hollow-cylinder and hollow-screw implants. *Clin Oral Implants Res* 1(1):33-40.

Buser D, Weber HP, Donath K, Fiorellini JP, Paquette DW, Williams RC (1992). Soft tissue reactions to non-submerged unloaded titanium implants in beagle dogs. *J Periodontol* 63(3):225-35.

Buser D, Mericske-Stern R, Bernard JP, Behneke A, Behneke N, Hirt HP, et al. (1997). Long-term evaluation of non-submerged ITI implants. Part 1: 8-year life table analysis of a prospective multi-center study with 2359 implants. *Clin Oral Implants Res* 8(3):161-72.

Caffesse RG, Mota LF, Morrison EC (1995). The rationale for periodontal therapy. *Periodontol 2000* 9(7-13.

Caton J, Nyman S, Zander H (1980). Histometric evaluation of periodontal surgery. II. Connective tissue attachment levels after four regenerative procedures. *J Clin Periodontol* 7(3):224-31.

Caton JG, Greenstein G (1993). Factors related to periodontal regeneration. *Periodontol 2000* 1(9-15.

Centty IG, Blank LW, Levy BA, Romberg E, Barnes DM (1997). Carbon dioxide laser for de-epithelialization of periodontal flaps. *J Periodontol* 68(8):763-9.

Cobb CM, McCawley TK, Killoy WJ (1992). A preliminary study on the effects of the Nd:YAG laser on root surfaces and subgingival microflora *in vivo*. *J Periodontol* 63(8):701-7.

Cochran DL, Hermann JS, Schenk RK, Higginbottom FL, Buser D (1997). Biologic width around titanium implants. A histometric analysis of the implanto-gingival junction around unloaded and loaded nonsubmerged implants in the canine mandible. *J Periodontol* 68(2):186-98.

Cree IA, Pazzagli M, Mini E, Mazzei T, Hunter EM, Sutherland LA, et al. (1995). Methotrexate chemosensitivity by ATP luminescence in human leukemia cell lines and in breast cancer primary cultures: comparison of the TCA-100 assay with a clonogenic assay. *Anticancer Drugs* 6(3):398-404.

Crouch SP, Kozlowski R, Slater KJ, Fletcher J (1993). The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. *J Immunol Methods* 160(1):81-8.

Dahlin C, Linde A, Gottlow J, Nyman S (1988). Healing of bone defects by guided tissue regeneration. *Plast Reconstr Surg* 81(5):672-6.

Eberhard J, Ehlers H, Falk W, Acil Y, Albers HK, Jepsen S (2003). Efficacy of subgingival calculus removal with Er:YAG laser compared to mechanical debridement: an in situ study. *J Clin Periodontol* 30(6):511-8.

Ericsson I, Berglundh T, Marinello C, Liljenberg B, Lindhe J (1992). Long-standing plaque and gingivitis at implants and teeth in the dog. *Clin Oral Implants Res* 3(3):99-103.

Ericsson I, Persson LG, Berglundh T, Edlund T, Lindhe J (1996). The effect of antimicrobial therapy on periimplantitis lesions. An experimental study in the dog. *Clin Oral Implants Res* 7(4):320-8.

Fartash B, Arvidson K, Ericsson I (1990). Histology of tissues surrounding single crystal sapphire endosseous dental implants: an experimental study in the beagle dog. *Clin Oral Implants Res* 1(1):13-21.

Featherstone JDB (2000). Caries detection and prevention with laser energy. *Dent Clin North Am* 44(955-969.

Folwaczny M, Mehl A, Haffner C, Benz C, Hickel R (2000). Root substance removal with Er:YAG laser radiation at different parameters using a new delivery system. *J Periodontol* 71(2):147-55.

Folwaczny M, Thiele L, Mehl A, Hickel R (2001). The effect of working tip angulation on root substance removal using Er:YAG laser radiation: an *in vitro* study. *J Clin Periodontol* 28(3):220-6.

Folwaczny M, Heym R, Mehl A, Hickel R (2002a). Subgingival calculus detection with fluorescence induced by 655 nm InGaAsP diode laser radiation. *J Periodontol* 73(6):597-601.

Folwaczny M, Mehl A, Aggstaller H, Hickel R (2002b). Antimicrobial effects of 2.94 microm Er:YAG laser radiation on root surfaces: an *in vitro* study. *J Clin Periodontol* 29(1):73-8.

Folwaczny M, Aggstaller H, Mehl A, Hickel R (2003). Removal of bacterial endotoxin from root surface with Er:YAG laser. *Am J Dent* 16(1):3-5.

Fox SC, Moriarty JD, Kusy RP (1990). The effects of scaling a titanium implant surface with metal and plastic instruments: an *in vitro* study. *J Periodontol* 61(8):485-90.

Gatewood RR, Cobb CM, Killoy WJ (1993). Microbial colonization on natural tooth structure compared with smooth and plasma-sprayed dental implant surfaces. *Clin Oral Implants Res* 4(2):53-64.

Gotfredsen K, Rostrup E, Hjorting-Hansen E, Stoltze K, Budtz-Jorgensen E (1991). Histological and histomorphometrical evaluation of tissue reactions adjacent to endosteal implants in monkeys. *Clin Oral Implants Res* 2(1):30-7.

Greenstein G, Caton JG (1993). Biodegradable barriers and guided tissue regeneration. *Periodontol 2000* 1(36-45.

Hahn R, Netuschil L, Löst C (1992). Initiale Plaquebesiedlung auf keramischen Restaurationsmaterialien. *Dtsch zahnärztl Z* 47(330-334.

Hale GM, Querry MR (1973). Optical constants of Water in the 200-nm to 200-µm wavelength region. *Appl Optics* 12(555-563.

Hardwick R, Scantlebury TV, Sanchez R, Whitley N, Ambruster J (1994). Guided bone regeneration in implant dentistry. *Quintessenz Pub.*

Hürzeler MB, Quinones CR, Schupback P, Morrison EC, Caffesse RG (1997). Treatment of peri-implantitis using guided bone regeneration and bone grafts, alone or in combination, in beagle dogs. Part 2: Histologic findings. *Int J Oral Maxillofac Implants* 12(2):168-75.

Hutmacher D, Hürzeler MB, Schliephake H (1996). A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications. *Int J Oral Maxillofac Implants* 11(5):667-78.

Isidor F, Karring T (1986). Long-term effect of surgical and non-surgical periodontal treatment. A 5-year clinical study. *J Periodontal Res* 21(5):462-72.

Israel M, Cobb CM, Rossmann JA, Spencer P (1997). The effects of CO2, Nd:YAG and Er:YAG lasers with and without surface coolant on tooth root surfaces. An *in vitro* study. *J Clin Periodontol* 24(9):595-602.

Kaldahl WB, Kalkwarf KL, Patil KD, Molvar MP, Dyer JK (1996). Long-term evaluation of periodontal therapy: I. Response to 4 therapeutic modalities. *J Periodontol* 67(2):93-102.

Karoussis IK, Salvi GE, Heitz-Mayfield LJ, Bragger U, Hämmerle CH, Lang NP (2003). Long-term implant prognosis in patients with and without a history of chronic periodontitis: a 10-year prospective cohort study of the ITI Dental Implant System. *Clin Oral Implants Res* 14(3):329-39.

Karoussis IK, Bragger U, Salvi GE, Burgin W, Lang NP (2004). Effect of implant design on survival and success rates of titanium oral implants: a 10-year prospective cohort study of the ITI Dental Implant System. *Clin Oral Implants Res* 15(1):8-17.

Kato T, Kusakari H, Hoshino E (1998). Bactericidal efficacy of carbon dioxide laser against bacteria-contaminated titanium implant and subsequent cellular adhesion to irradiated area. *Lasers Surg Med* 23(5):299-309.

Kimura Y, Yu DG, Fujita A, Yamashita A, Murakami Y, Matsumoto K (2001a). Effects of erbium,chromium:YSGG laser irradiation on canine mandibular bone. *J Periodontol* 72(9):1178-82.

Kimura Y, Yu DG, Kinoshita J, Hossain M, Yokoyama K, Murakami Y, et al. (2001b). Effects of erbium, chromium:YSGG laser irradiation on root surface: morphological and atomic analytical studies. *J Clin Laser Med Surg* 19(2):69-72.

Kinane DF (2001). Causation and pathogenesis of periodontal disease. *Periodontol 2000* 25(8-20.

Kohal RJ, Mellas P, Hurzeler MB, Trejo PM, Morrison E, Caffesse RG (1998). The effects of guided bone regeneration and grafting on implants placed into immediate extraction sockets. An experimental study in dogs. *J Periodontol* 69(8):927-37.

Krause F, Braun A, Frentzen M (2003). The possibility of detecting subgingival calculus by laser-fluorescence *in vitro*. *Lasers Med Sci* 18(1):32-5.

Kreisler M, Al Haj H, Daubländer M, Gotz H, Duschner H, Willershausen B, et al. (2002a). Effect of diode laser irradiation on root surfaces *in vitro*. *J Clin Laser Med Surg* 20(2):63-9.

Kreisler M, Al Haj H, Gotz H, Duschner H, d'Hoedt B (2002b). Effect of simulated CO(2) and GaAlAs laser surface decontamination on temperature changes in Ti-plasma sprayed dental implants. *Lasers Surg Med* 30(3):233-9.

Kreisler M, Gotz H, Duschner H (2002c). Effect of Nd:YAG, Ho:YAG, Er:YAG, CO2, and GaAIAs laser irradiation on surface properties of endosseous dental implants. *Int J Oral Maxillofac Implants* 17(2):202-11.

Kreisler M, Kohnen W, Marinello C, Gotz H, Duschner H, Jansen B, et al. (2002d). Bactericidal effect of the Er:YAG laser on dental implant surfaces: an *in vitro* study. *J Periodontol* 73(11):1292-8.

Kreisler M, Kohnen W, Christoffers AB, Gotz H, Jansen B, Duschner H, et al. (2005). *In vitro* evaluation of the biocompatibility of contaminated implant surfaces treated with an Er : YAG laser and an air powder system. *Clin Oral Implants Res* 16(1):36-43.

Lang NP, Brägger U, Walther D, Beamer B, Kornman KS (1993). Ligature-induced peri-implant infection in cynomolgus monkeys. I. Clinical and radiographic findings. *Clin Oral Implants Res* 4(1):2-11.

Leonhardt A, Olsson J, Dahlen G (1995). Bacterial colonization on titanium, hydroxyapatite, and amalgam surfaces *in vivo*. *J Dent Res* 74(9):1607-12.

Leonhardt A, Renvert S, Dahlen G (1999). Microbial findings at failing implants. *Clin Oral Implants Res* 10(5):339-45.

Lide DR (2002). Handbook of Chemistry and Physics. *CRC Press*: 83rd edition.

Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C (1992). Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. *Clin Oral Implants Res* 3(1):9-16.

Lindhe J, Berglundh T (1998). The interface between the mucosa and the implant. *Periodontol 2000* 17(47-54.

Listgarten MA, Buser D, Steinemann SG, Donath K, Lang NP, Weber HP (1992). Light and transmission electron microscopy of the intact interfaces between non-submerged titanium-coated epoxy resin implants and bone or gingiva. *J Dent Res* 71(2):364-71.

Liu CM, Hou LT, Wong MY, Lan WH (1999). Comparison of Nd:YAG laser versus scaling and root planing in periodontal therapy. *J Periodontol* 70(11):1276-82.

Löe H (1967). The Gingival Index, the Plaque Index and the Retention Index Systems. *J Periodontol* 38(6):Suppl:610-6.

Maehara Y, Anai H, Tamada R, Sugimachi K (1987). The ATP assay is more sensitive than the succinate dehydrogenase inhibition test for predicting cell viability. *Eur J Cancer Clin Oncol* 23(3):273-6.

Magnusson I, Batich C, Collins BR (1988). New attachment formation following controlled tissue regeneration using biodegradable membranes. *J Periodontol* 59(1):1-6.

Marinello CP, Berglundh T, Ericsson I, Klinge B, Glantz PO, Lindhe J (1995). Resolution of ligature-induced peri-implantitis lesions in the dog. *J Clin Periodontol* 22(6):475-9.

Matarasso S, Quaremba G, Coraggio F, Vaia E, Cafiero C, N.P. L (1996). Maintenance of implants: an *in vitro* study of titanium implant surface modifications, subsequent to the application of different peophylaxis procedures. *Clin Oral Implants Res* 7(64-72.

Matsuyama T, Aoki A, Oda S, Yoneyama T, Ishikawa I (2003). Effects of the Er:YAG laser irradiation on titanium implant materials and contaminated implant abutment surfaces. *J Clin Laser Med Surg* 21(1):7-17.

Miller RJ (2004). Treatment of the contaminated implant surface using the Er,Cr:YSGG laser. *Implant Dent* 13(2):165-70.

Mombelli A, van Oosten MA, Schurch E, Jr., Land NP (1987). The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiol Immunol* 2(4):145-51.

Mombelli A, Buser D, Lang NP (1988). Colonization of osseointegrated titanium implants in edentulous patients. Early results. *Oral Microbiol Immunol* 3(3):113-20.

Mombelli A, Lang NP (1992). Antimicrobial treatment of peri-implant infections. *Clin Oral Implants Res* 3(4):162-8.

Moritz A, Schoop U, Goharkhay K, Schauer P, Doertbudak O, Wernisch J, et al. (1998). Treatment of periodontal pockets with a diode laser. *Lasers Surg Med* 22(5):302-11.

Mouhyi J, Sennerby L, Wennerberg A, Louette P, Dourov N, van Reck J (2000). Re-establishment of the atomic composition and the oxide structure of contaminated titanium surfaces by means of carbon dioxide laser and hydrogen peroxide: an *in vitro* study. *Clin Implant Dent Relat Res* 2(4):190-202.

Murray E, Provvedini D, Curran D, Catherwood B, Sussman H, Manolagas S (1987). Characterization of a human osteoblastic osteosarcoma cell line (SAOS-2) with high bone alkaline phosphatase activity. *J Bone Miner Res* 2(3):231-8.

Nociti FH, Jr., Machado MA, Stefani CM, Sallum EA (2001). Absorbable versus nonabsorbable membranes and bone grafts in the treatment of ligature-induced peri-implantitis defects in dogs: a histometric investigation. *Int J Oral Maxillofac Implants* 16(5):646-52.

O'Leary TJ (1986). The impact of research on scaling and root planing. *J Periodontol* 57(2):69-75.

Oyster DK, Parker WB, Gher ME (1995). CO2 lasers and temperature changes of titanium implants. *J Periodontol* 66(12):1017-24.

Persson LG, Ericsson I, Berglundh T, Lindhe J (1996). Guided bone regeneration in the treatment of periimplantitis. *Clin Oral Implants Res* 7(4):366-72.

Petty RD, Sutherland LA, Hunter EM, Cree IA (1995). Comparison of MTT and ATP-based assays for the measurement of viable cell number. *J Biolumin Chemilumin* 10(1):29-34.

Polson AM, Frederick GT, Ladenheim S, Hanes PJ (1984). The production of a root surface smear layer by instrumentation and its removal by citric acid. *J Periodontol* 55(8):443-6.

Pontoriero R, Tonelli MP, Carnevale G, Mombelli A, Nyman SR, Lang NP (1994). Experimentally induced peri-implant mucositis. A clinical study in humans. *Clin Oral Implants Res* 5(4):254-9.

Postlethwaite AE, Seyer JM, Kang AH (1978). Chemotactic attraction of human fibroblasts to type I, II, and III collagens and collagen-derived peptides. *Proceedings of the National Academy of Sciences of the United States of America* 75(2):871-5.

Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Arends J, Darius PL, et al. (1989). The influence of surface free-energy on planimetric plaque growth in man. *J Dent Res* 68(5):796-9.

Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, van Steenberghe D (1990). The influence of surface free energy and surface roughness on early plaque formation. An *in vivo* study in man. *J Clin Periodontol* 17(3):138-44.

Quirynen M, van der Mei HC, Bollen CM, Schotte A, Marechal M, Doornbusch GI, et al. (1993). An *in vivo* study of the influence of the surface roughness of implants on the microbiology of supra- and subgingival plaque. *J Dent Res* 72(9):1304-9.

Quirynen M, van Steenberghe D (1993). Bacterial colonization of the internal part of two-stage implants. An *in vivo* study. *Clin Oral Implants Res* 4(3):158-61.

Quirynen M, Bollen CM, Eyssen H, van Steenberghe D (1994). Microbial penetration along the implant components of the Branemark system. An *in vitro* study. *Clin Oral Implants Res* 5(4):239-44.

Quirynen M, Bollen CM, Papaioannou W, Van Eldere J, van Steenberghe D (1996). The influence of titanium abutment surface roughness on plaque accumulation and gingivitis: short-term observations. *Int J Oral Maxillofac Implants* 11(2):169-78.

Ramfjord SP, Caffesse RG, Morrison EC, Hill RW, Kerry GJ, Appleberry EA, et al. (1987). Four modalities of periodontal treatment compared over five years. *J Periodontal Res* 22(3):222-3.

Rams TE, Roberts TW, Tatum H, Jr., Keyes PH (1984). The subgingival microbial flora associated with human dental implants. *J Prosthet Dent* 51(4):529-34.

Rams TE, Roberts TW, Feik D, Molzan AK, Slots J (1991). Clinical and microbiological findings on newly inserted hydroxyapatite-coated and pure titanium human dental implants. *Clin Oral Implants Res* 2(3):121-7.

Rechmann P, Sadegh HM, Goldin DS, Hennig TH (2000). Zur Oberflächenmorphologie von Implantaten nach Laserbestrahlung. *Dtsch Zahnärztl Z* 55(371-376.

Rimondini L, Fare S, Brambilla E, Felloni A, Consonni C, Brossa F, et al. (1997). The effect of surface roughness on early *in vivo* plaque colonization on titanium. *J Periodontol* 68(6):556-62.

Robertson CW, Williams D (1971). Lambert absorption coefficients of water in the infrared. *J Opt Soc Am* 61(1316-1320.

Rodan SB, Imai Y, Thiede MA, Wesolowski G, Thompson D, Bar-Shavit Z, et al. (1987). Characterization of a human osteosarcoma cell line (Saos-2) with osteoblastic properties. *Cancer Res* 47(18):4961-6.

Romanos GE, Everts H, Nentwig GH (2000). Effects of diode and Nd:YAG laser irradiation on titanium discs: a scanning electron microscope examination. *J Periodontol* 71(5):810-5.

Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J (2005). Biodegradation of differently cross-linked collagen membranes. An experimental study in the rat. *Clin Oral Implants Res* 16(3):369-678.

Rühling A, Kocher T, Kreusch J, Plagmann HC (1994). Treatment of subgingival implant surfaces with Teflon-coated sonic and ultrasonic scaler tips and various implant curettes. An *in vitro* study. *Clin Oral Implants Res* 5(1):19-29.

Schenk G, Flemmig TF, Betz T, Reuther J, Klaiber B (1997). Controlled local delivery of tetracycline HCl in the treatment of periimplant mucosal hyperplasia and mucositis. A controlled case series. *Clin Oral Implants Res* 8(5):427-33.

Schou S, Holmstrup P, Stoltze K, Hjorting-Hansen E, Kornman KS (1993). Ligature-induced marginal inflammation around osseointegrated implants and ankylosed teeth. Clinical and radiographic observations in Cynomolgous monkeys. *Clin Oral Implants Res* 4(1):12-22.

Schou S, Holmstrup P, Jorgensen T, Skovgaard LT, Stoltze K, Hjorting-Hansen E, et al. (2003a). Anorganic porous bovine-derived bone mineral (Bio-Oss) and ePTFE membrane in the treatment of peri-implantitis in cynomolgus monkeys. *Clin Oral Implants Res* 14(5):535-47.

Schou S, Holmstrup P, Jorgensen T, Skovgaard LT, Stoltze K, Hjorting-Hansen E, et al. (2003b). Implant surface preparation in the surgical treatment of experimental peri-implantitis with autogenous bone graft and ePTFE membrane in cynomolgus monkeys. *Clin Oral Implants Res* 14(4):412-22.

Schou S, Holmstrup P, Skovgaard LT, Stoltze K, Hjorting-Hansen E, Gundersen HJ (2003c). Autogenous bone graft and ePTFE membrane in the treatment of peri-implantitis. II. Stereologic and histologic observations in cynomolgus monkeys. *Clin Oral Implants Res* 14(4):404-11.

Schou S, Berglundh T, Lang NP (2004). Surgical treatment of peri-implantitis. *Int J Oral Maxillofac Implants* 19 Suppl(140-9.

Schroeder HE (1965). Crystal Morphology and Gross Structures of Mineralizing Plaque and of Calculus. *Helv Odontol Acta* 35(73-86.

Schwarz F, Sculean A, Arweiler N, Reich E (2000). Nicht-chirurgische Parodontalbehandlung mit einem Er:YAG Laser. *Parodontologie* 4(329-336.

Schwarz F, Sculean A, Georg T, Reich E (2001). Periodontal treatment with an Er: YAG laser compared to scaling and root planing. A controlled clinical study. *J Periodontol* 72(3):361-7.

Schwarz F, Rothamel D, Becker J (2003a). Influence of an Er:YAG laser on the surface structure of titanium implants. *Schweiz Monatsschr Zahnmed* 113(6):660-71.

Schwarz F, Rothamel D, Sculean A, Georg T, Scherbaum W, Becker J (2003b). Effects of an Er:YAG laser and the Vector ultrasonic system on the biocompatibility of titanium implants in cultures of human osteoblast-like cells. *Clin Oral Implants Res* 14(6):784-92.

Schwarz F, Sculean A, Berakdar M, Georg T, Reich E, Becker J (2003c). Periodontal treatment with an Er:YAG laser or scaling and root planing. A 2-year follow-up split-mouth study. *J Periodontol* 74(5):590-6.

Schwarz F, Sculean A, Berakdar M, Georg T, Reich E, Becker J (2003d). Clinical evaluation of an Er:YAG laser combined with scaling and root planing for non-surgical periodontal treatment. A controlled, prospective clinical study. *J Clin Periodontol* 30(1):26-34.

Schwarz F, Sculean A, Berakdar M, Szathmari L, Georg T, Becker J (2003e). *In vivo* and *in vitro* effects of an Er:YAG laser, a GaAlAs diode laser, and scaling and root planing on periodontally diseased root surfaces: A comparative histologic study. *Lasers Surg Med* 32(5):359-366.

Schwarz F, Sculean A, Georg T, Becker J (2003f). Clinical evaluation of the Er:YAG laser in combination with an enamel matrix protein derivative for the treatment of intrabony periodontal defects: A pilot study. *J Clin Periodontol* 30(11):975-81.

Schwarz F, Rothamel D, Herten M, Bieling K, Scherbaum W, Becker J (2004). Effects of an Er:YAG laser on mitochondrial activity of human osteosarcoma-derived osteoblasts *in vitro*. *Lasers Med Sci* 19(1):37-40.

Schwarz F, Sculean A, Rothamel D, Schwenzer K, Georg T, Becker J (2005a). Clinical evaluation of an Er:YAG laser for nonsurgical treatment of peri-implantitis. A pilot study. *Clin oral Implants Res* 16(1):44-52.

Schwarz F, Sculean A, Romanos G, Herten M, Horn N, Scherbaum W, et al. (2005b). Influence of different treatment approaches on the removal of early plaque biofilms and the viability of SAOS2 osteoblasts grown on titanium implants. *Clin Oral Investig* 9(2):111-7.

Schwarz F, Papanicolau P, Rothamel D, Beck B, Herten M, Becker J (2006a). Influence of plaque biofilm removal on reestablishment of the biocompatibility of contaminated titanium surfaces. *J Biomed Mater Res* 77(3):437-44.

Schwarz F, Rothamel D, Herten M, Sager M, Becker J (2006b). Angiogenesis pattern of native and cross-linked collagen membranes. An immunohistochemical study in the rat. *Clin Oral Implants Res* 17(4):403-9.

Sculean A, Donos N, Brecx M, Reich E, Karring T (2000). Treatment of intrabony defects with guided tissue regeneration and enamel-matrix-proteins. An experimental study in monkeys. *J Clin Periodontol* 27(7):466-72.

Sculean A, Windisch P, Keglevich T, Gera I (2003). Histologic evaluation of human intrabony defects following non-surgical periodontal therapy with and without application of an enamel matrix protein derivative. *J Periodontol* 74(2):153-60.

Sculean A, Schwarz F, Berakdar M, Windisch P, Arweiler NB, Romanos GE (2004). Healing of intrabony defects following surgical treatment with or without an Er:YAG laser. *J Clin Periodontol* 31(8):604-8.

Sela MN, Kohavi D, Krausz E, Steinberg D, Rosen G (2003). Enzymatic degradation of collagen-guided tissue regeneration membranes by periodontal bacteria. *Clin Oral Implants Res* 14(3):263-8.

Sennerby L, Lekholm U (1993). The soft tissue response to titanium abutments retrieved from humans and reimplanted in rats. A light microscopic study. *Clin Oral Implants Res* 4(1):23-7.

Siegrist BE, Brecx MC, Gusberti FA, Joss A, Lang NP (1991). *In vivo* early human dental plaque formation on different supporting substances. A scanning electron microscopic and bacteriological study. *Clin Oral Implants Res* 2(1):38-46.

Sugi D, Fukuda M, Minoura S, Yamada Y, Tako J, Miwa K, et al. (1998). Effects of irradiation of Er:YAG laser on quantity of endotoxin and microhardness of surface in exposed root after removal of calculus. *Jp J Cons Dent* 41(1009-1017.

Tatakis DN, Promsudthi A, Wikesjö UM (1999). Devices for periodontal regeneration. *Periodontol 2000* 19(59-73.

Tewfik HM, Garnick JJ, Schuster GS, Sharawy MM (1994). Structural and functional changes of cementum surface following exposure to a modified Nd:YAG laser. *J Periodontol* 65(4):297-302.

Theodoro LH, Haypek P, Bachmann L, Garcia VG, Sampaio JE, Zezell DM, et al. (2003). Effect of ER:YAG and diode laser irradiation on the root surface: morphological and thermal analysis. *J Periodontol* 74(6):838-43.

Tucker D, Cobb CM, Rapley JW, Killoy WJ (1996). Morphologic changes following *in vitro* CO2 laser treatment of calculus-ladened root surfaces. *Lasers Surg Med* 18(2):150-6.

Van de Velde E, Thielens P, Schautteet H, Vanclooster R (1991). Subcutaneous emphysema of the oral floor during cleaning of a bridge fixed on an IMZ implant. Case report. *Rev Belge Med Dent* 46(3):64-71.

van Steenberghe D, Klinge B, Linden U, Quirynen M, Herrmann I, Garpland C (1993). Periodontal indices around natural and titanium abutments: a longitudinal multicenter study. *J Periodontol* 64(6):538-41.

van Steenberghe D (1997). Outcomes and their measurement in clinical trials of endosseous oral implants. *Ann Periodontol* 2(1):291-8.

Wang X, Zhang C, Matsumoto K (2005). *In vivo* study of the healing processes that occur in the jaws of rabbits following perforation by an Er,Cr:YSGG laser. *Lasers Med Sci* 20(1):21-7.

Watanabe H, Ishikawa I, Suzuki M, Hasegawa K (1996). Clinical assessments of the erbium:YAG laser for soft tissue surgery and scaling. *J Clin Laser Med Surg* 14(2):67-75.

Wilder-Smith P, Arrastia AM, Schell MJ, Liaw LH, Grill G, Berns MW (1995). Effect of ND:YAG laser irradiation and root planing on the root surface: structural and thermal effects. *J Periodontol* 66(12):1032-9.

Yaffe A, Ehrlich J, Shoshan S (1984). Restoration of periodontal attachment employing enriched collagen solution in the dog. *J Periodontol* 55(11):623-8.

Yamaguchi H, Kobayashi K, Osada R, Sakuraba E, Nomura T, Arai T, et al. (1997). Effects of irradiation of an erbium:YAG laser on root surfaces. *J Periodontol* 68(12):1151-5.

Zitzmann NU, Abrahamsson I, Berglundh T, Lindhe J (2002). Soft tissue reactions to plaque formation at implant abutments with different surface topography. An experimental study in dogs. *J Clin Periodontol* 29(5):456-61.

Zitzmann NU, Berglundh T, Ericsson I, Lindhe J (2004). Spontaneous progression of experimentally induced periimplantitis. *J Clin Periodontol* 31(10):845-9.

LEBENSLAUF

Persönliche Daten

Name:	Enaas Nuesry
Geburtsdatum:	14.5.1974, Ohio - USA
E-mail-Adresse:	enaasnuesry@med.uni-duesseldorf.de
Familienstand:	ledig
Staatsangehörigkeit:	Lybien / USA
Eltern:	
Mutter:	PhD. Fawzia Sikta, Mitarbeiterin an der Al-Fateh Universität, Tripolis
Vater:	Prof. Dr. Saleh Nuesry, Mitarbeiter an der Al-Fateh Universitat,Tripolis

Berufserfahrungen

Seit Okt 2004	Anstellung als Gastzahnärztin an der Universitätsklinik Düsseldorf, Westdeutsche Kieferklinik, Abteilung für Zahnärztliche Chirurgie und Aufnahme
2000 - 2003	Wissenschaftliche Mitarbeiterin an der Universitätsklinik, Abteilung für Oralchirurgie, Al-Fateh-Universität, Tripolis, Libyen
2002 - 2003	Assistentenzzahnärztin in der Poliklinik Mwasat, Tripolis, Libyen
2000 - 2002	Assistentenzzahnärztin in der Privatpraxis Ekaa, Tripolis, Libyen
1999 - 2000	1-jähriger obligatorischer Dienst in der Zentralen Zahnklinik, Tripolis, Libyen
1998 - 1999	1-praktisches Jahr in der Uniklinik, Tripolis, Libyen

Schulen/Ausbildung

2003 - 2004	Deutsch-Sprachkurs am Goethe-Institut, Düsseldorf, Deutschland
1992 - 1998	Studium der Zahnmedizin an der Al-Fateh-Universität, Tripolis, Libyen. Abschlussnote: sehr gut
1989 - 1992	Besuch des Gymnasiums Tripolis, Tripoli, Libyen (Abitur)
1982 - 1989	Besuch der Grundschule Garnata, Tripolis, Libyen

Publikationen

Journal of Clinical Periodontology (2006, 33: 491-499)
Schwarz F., Bieling K., Latz T., Nuesry E., Becker J.
Healing of intrabony peri-implantitis defects following application of a nanocristalline hydroxyapatite (Ostim) or a bovine-derived xenograft (Bio-Oss) in combination with a collagen membrane (Bio-Gide)

Lasers in Surgery and Medicine (2006, 38: 663-671)
Schwarz F., Bieling K., Nuesry E., Sculean A., Becker J.
Clinical and histological healing pattern of peri-implantitis lesions following nonsurgical treatment with an Er:YAG laser

Journal of Periodontology (2006, 67: 1820-1827)
Schwarz F., Nuesry E., Bieling K., Herten M., Becker J.
Influence of an Er,Cr:YSGG laser on the re-establishment of the biocompatibility of contaminated titanium implant surfaces