Clin Oral Invest (2005) 9: 111–117
DOI 10.1007/s00784-005-0305-8

ORIGINAL ARTICLE

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Influence of different treatment approaches on the removal of early plaque biofilms and the viability of SAOS2 osteoblasts grown on titanium implants

Received: 24 May 2004 / Accepted: 15 December 2004 / Published online: 20 April 2005

Abstract The aim of the present study was to evaluate the influence of different treatment approaches on: (1) the removal of early plaque biofilms grown on titanium implants, and (2) the biocompatibility of the instrumented implant surfaces. Five volunteers wore acrylic splints with sandblasted and acid-etched titanium discs for 24 h to build up supragingival plaque. A total of 80 specimens were randomly assigned to the following groups: (1) an Er:YAG laser (100 mJ/pulse, 10 Hz) (Y), (2) an ultrasonic system (U), (3) plastic curettes and rinsing with chlorhexidine digluconate (P), or (4) unworn titanium discs (C). Autoclaved specimens were incubated with SAOS2 cells for three days. The following parameters were measured: treatment time (T), residual plaque biofilm (RPB) and clean implant surface (CIS) areas (%), and mitochondrial cell activity (MA) (counts/s). Statistical analysis within and between groups revealed the following mean scores (±SD): RPB areas: P (61.1±11.4) > U (36.8±4.5) > Y (5.8±5.1); CIS areas: Y (94.2±5.1) > U (63.2±4.5) > P (38.9±11.2); T: Y (5.6±1.2) > U (2.4±0.5) > P (2.3±0.5); MA: C (1.528.636±188.371) > U (831.594±370.228) > Y (678.250±367.902) > P (144.105±120.961). Within the limits of the present study, it may be concluded that Y seems to be most suitable for the removal of supragingival early plaque biofilms grown on SLA titanium implants, and (2) all treatment procedures failed to restore the biocompatibility of previously-contaminated SLA titanium surfaces.

Keywords Biofilm model · Titanium discs · Er:YAG laser · Ultrasonic system · Cell culture

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Introduction

Nowadays, microbial colonization has been implicated as the main causative factor in the pathogenesis of implant failures [3, 8, 23]. Bacteria present on implant surfaces may lead to an inflammation of the peri-implant mucosa, and, if left untreated, the inflammation spreads apically and results in bone loss, a process that has been named peri-implantitis [2]. Therefore, the removal of bacterial biofilms seems to be a prerequisite in the therapy of peri-implant infections [25]. However, decontamination of structured implant surfaces is difficult to achieve. Since mechanical methods (plastic curettes, polishing with rubber cups) alone have been proven to be insufficient in the elimination of bacteria on roughened implant surfaces, the adjunctive use of chemical agents (irrigation with local disinfectants, local or systemic antibiotic therapy) has been recommended in order to enhance healing following treatment [12, 24, 34]. Furthermore, air-powder-flow was also successfully used for implant surface decontamination in vitro [4, 28]. However, there are limitations in their application because they can be associated with an increased risk of emphysema [43]. Recently, in addition to these conventional tools, the use of different laser systems has been proposed for the decontamination of implant surfaces [17, 18, 27, 33]. Recent in vitro studies have pointed out that (in an energy-dependent manner) only the CO_2 (carbon-dioxide) laser, the diode laser and the Er:YAG (erbium-doped: yttrium, aluminium and garnet) laser may be suitable for the irradiation of implant surfaces because their specific wavelengths are

poorly absorbed by titanium and so the implant body temperature does not increase significantly during irradiation [16–19, 27, 33]. However, until now, bactericidal effects on roughened implant surfaces in vitro have only been reported for the CO_2− and Er:YAG laser [16, 19]. Since neither CO_2 nor diode lasers are effective at removing plaque biofilms from root surfaces or titanium implants, both types of lasers were only used in conjunction with mechanical treatment procedures [6, 26, 38, 42]. In contrast, the ability of the Er:YAG laser to effectively ablate dental calculus from periodontally-diseased root surfaces without producing thermal side-effects to adjacent tissue has been demonstrated in recent in vivo studies [11, 38]. Preliminary results from a controlled clinical trial have also indicated that nonsurgical treatment of peri-implantitis with an Er:YAG laser may lead to significant clinical improvements, as evidenced by bleeding on probing, probing depth reduction and gain of clinical attachment [39]. However, until now, no investigations evaluating the influence of an Er:YAG laser on the removal of plaque biofilms from rough titanium implants have been available. Therefore, the aim of the present study was to evaluate the effectiveness of an Er:YAG laser at removing plaque biofilms grown on titanium implants in comparison to conventional treatment procedures such as plastic curettes and rinsing with chlorhexidine digluconate or an ultrasonic system. Furthermore, the biocompatibility of the instrumented titanium surfaces was assessed through viability measurements of human osteoblast-like cells (SAOS2).

Materials and methods

Study population

Five healthy volunteers (three women, two men, mean age 31.5±1.8 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 ethics committee. The criteria needed for inclusion were: (1) no systemic use of antibiotics during the last six months, (2) good level of oral hygiene (PI<1) [20], (3) no signs of destructive periodontitis or any inflammatory conditions of the surrounding soft tissues, and (4) non-smoker.

Splints and titanium discs

Prior to the investigation, the subjects received a professional tooth cleaning. The volunteers were provided with acrylic appliances for the upper jaw with eight titanium discs (Promote, Altatec, Wimsheim, Germany; sand-blasted and acid-etched, 0.7 cm^2, 2-mm thick) each in order to collect a supragingival plaque biofilm [14]. Specimens were inserted with sticky wax into depressions towards the palate at a distance of approximately 1 mm, in order to provide a nutritious aqueous environment (Fig. 1). The splints were worn by the volunteers for 24 h [32, 41]. 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 toothbrushing (only with tap water, no toothpaste or mouth rinse was allowed). Erythrosine dye was used to stain the plaque biofilm grown on the implant surfaces. Only specimens exhibiting a homogenous plaque biofilm, as evidenced by light microscopic observation (Olympus BX50, Olympus, Hamburg, Germany, original magnification ×40), were included in the study. A total of 60 titanium discs were collected and equally and randomly assigned to the following treatment groups: (1) an Er:YAG laser (test group 1), (2) an ultrasonic system (test group 2), or (3) plastic curettes and rinsing with chlorhexidine digluconate (0.2%) (test group 3). Additionally, 20 unworn and untreated titanium discs served as positive control (C).

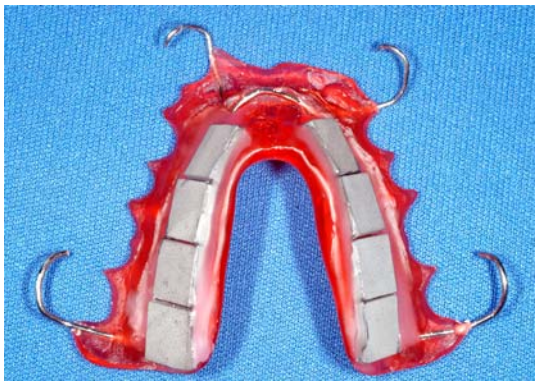

Fig. 1 Removable splints showing the location of the titanium discs in the upper jaw. Eight specimens were inserted into depressions with sticky wax towards the palate

Treatment procedures

An Er:YAG laser device (KEY3, KaVo, Biberach, Germany) emitting pulsed infrared radiation at a wavelength of

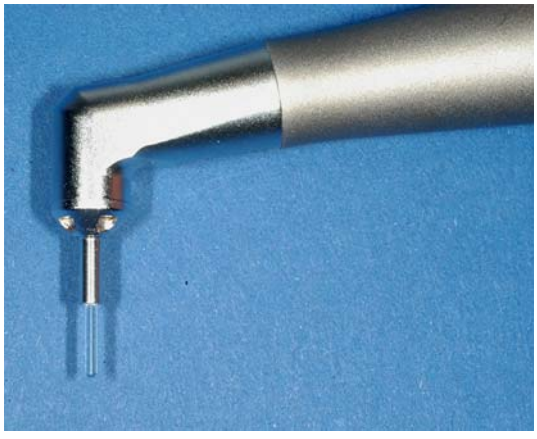

Fig. 2 Laser handpiece with a cone-shaped glass fibre tip emitting a radial and axial laser beam

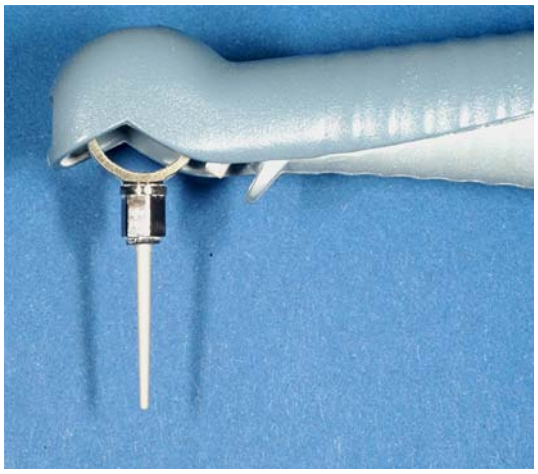

Fig. 3 Straight polyether ethercetone fibre of the Vector ultrasonic system

2.94 μm was selected for laser treatment (Y). Laser parameters were set at 100 mJ/pulse (12.7 J/cm^2), 10 Hz, and the pulse energy at the tip was approximately 85 mJ/pulse [36, 37, 39]. The laser beam was guided onto the implant surfaces under water irrigation with a specially-designed periodontal handpiece (P2061, KaVo, Biberach, Germany) and a cone-shaped glass fibre tip emitting a radial and axial laser beam (Fig. 2). For the treatment of test group 2, a specially-designed ultrasonic system was used (Vector, Dürr, Bietigheim-Bissingen, Germany) (U) with a straight polyether ethercetone fibre (PEEK) and a polishing fluid (HA particles<10 μm) (Fig. 3). In both groups, the fibre tips were guided parallel to the titanium surfaces in contact mode. In test group 3, the mechanical plaque biofilm removal was performed using plastic curettes (Straumann, Waldenburg, Switzerland) followed by irrigation with a 0.2% chlorhexidine digluconate solution (Corsodyl, GlaxoSmithKline Consumer Healthcare, Bühl, Germany) (P). The end point of the treatment was defined as the inability to remove residual plaque biofilm areas.

Measurement of residual plaque biofilm areas and clean implant surface areas

For image acquisition, a digital camera (Nikon D100, Nikon GmbH, Düsseldorf, Germany) was mounted on a binocular light microscope (Olympus BX50, Olympus, Hamburg, Germany). Digital images (original magnification ×40) were evaluated using a software program (ImageJ, Scion Corp., Maryland, USA). For each titanium disc, mean initial plaque biofilm (IPB) areas, residual plaque biofilm (RPB) areas and clean implant surface (CIS) areas were measured as a percentage of the scanned surface at ten fields selected at random. In order to calculate RPB and CSI areas, respective IPB areas were defined as 100%. All measurements were performed by one blinded and calibrated examiner.

Cell cultures

Subsequent to measurement of residual plaque areas, specimens were autoclaved and placed into 24-well plates (Lap Tek Chamber Slide, Nalge Nunc, Naperville, IL, USA). Human osteoblast-like SAOS2 cells (ATCC, No. HTB 85, Manassas, USA) [2×10^4 cells, fourth passage] were suspended in McCoy's 5A medium (MCM, Gibco No. 21017-025, Life Technologies GmbH, Karlsruhe, Germany) containing 1% penicillin/streptomycin (Gibco Invitrogen, Karlsruhe, Germany) and 10% fetal bovine serum (Gibco Invitrogen, Karlsruhe, Germany) and then inoculated onto the well chambers. Culturing was set at 37 °C in a humified atmosphere of 95% air and 5% CO_2. Twenty unworn titanium discs served as positive control (C).

Cell viability assay

At day 3, the mitochondrial activity in the SAOS2 osteoblasts was measured using the CellTiter-Glo luminescent cell viability assay (Promega, Madison, WI, USA). This assay quantifies the ATP present, which signals the presence of metabolically active cells and is based on the

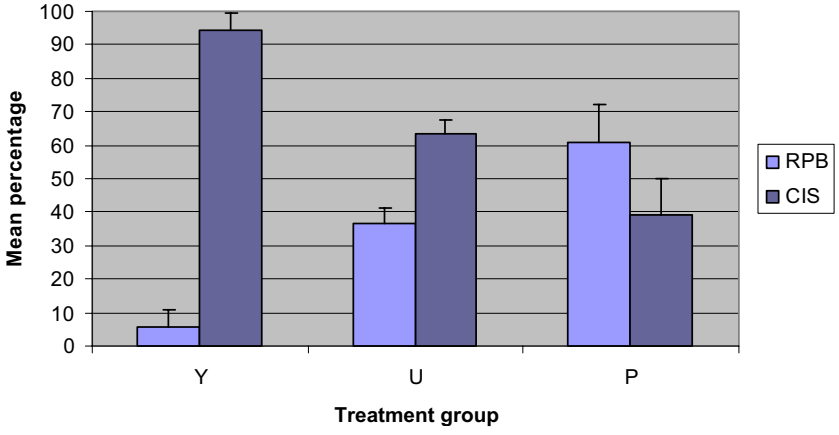

Fig. 4 Mean percent (±SD) residual plaque biofilm (RPB) areas and clean implant surface (CIS) areas in different treatment groups

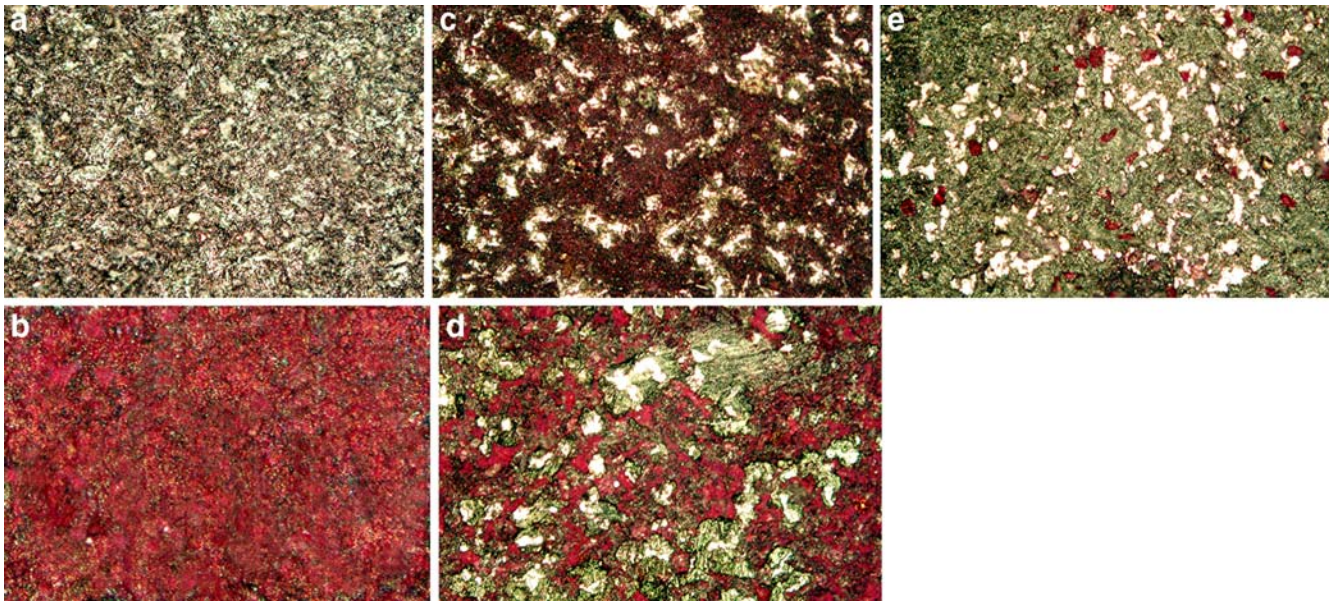

Fig. 5a–e Light microscopic view of residual plaque biofilm (RPB) areas and clean implant surface (CIS) areas (original magnification ×40): **a** control specimen, **b** homogeneously-stained plaque biofilm after 24 h, **c** RPB and CSI areas following treatment with P, **d** RPB and CSI areas following treatment with U, **e** RPB and CSI areas following treatment with Y

luciferase-catalysed reaction of luciferin and ATP. In particular, mono-oxygenation of luciferin is catalysed by luciferase in the presence of Mg^{2+}, ATP and molecular oxygen. One hundred microlitres of CellTiter-Glo reagent was added to the wells (n=80) and incubated for 10 min at room temperature. The luminescent signal was recorded for 1 s per well in a counter (Top Count, Canberra-Packard GmbH, Dreieich, Germany).

Statistical analysis

A software package (SPSS 11.0, SPSS Inc., Chicago, IL, USA) was used for the statistical analysis, defining the titanium discs as statistical units. Mean values and standard deviations were calculated for each group. Analysis of variance (ANOVA) and post hoc testing using Bonferroni's correction for multiple comparisons were used for comparisons within and between groups. Results were considered statistically significant at P<0.05.

Results

Residual plaque biofilm areas and clean implant surface areas

After 24 h, the following mean IPB areas were measured: Y: 97.8±0.9; U: 97.5±0.9; P: 97.4±1.0 (P>0.05, respectively). All treatment procedures resulted in a significant decrease in IPB area (P<0.001, respectively). The mean percent RPB and CIS areas for each treatment group are presented in Fig. 4 (light microscopic views of the RPB and CIS areas for each treatment group are shown in Fig. 5a–e). The highest

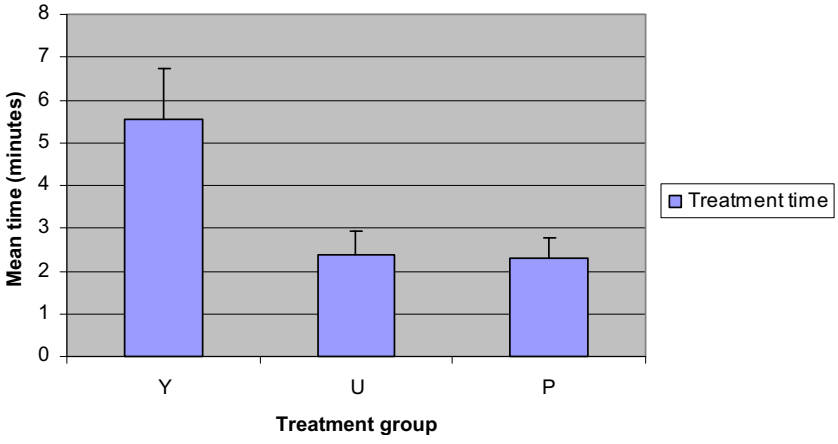

Fig. 6 Mean amount of time (min±SD) needed for implant surface instrumentation, defined as the inability to remove residual plaque biofilm in different treatment groups

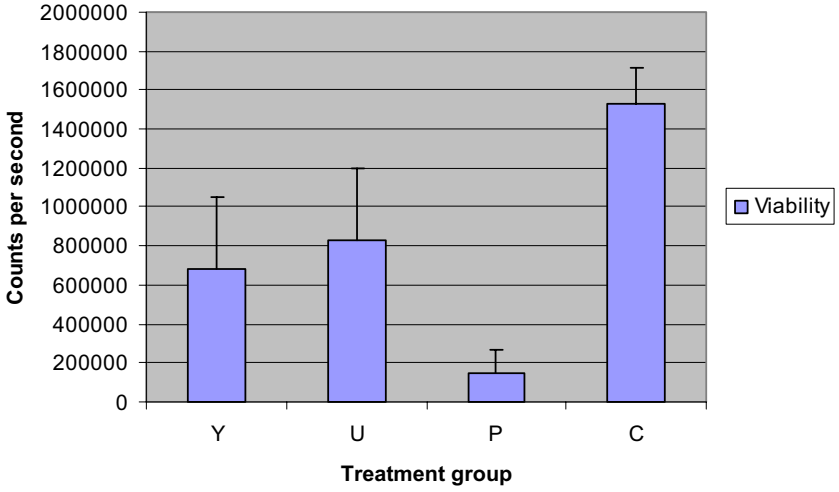

Fig. 7 CellTiter-Glo luminescent cell viability assay: mean mitochondrial activity (±SD) of SAOS2 osteoblasts (counts per second) in different groups

percent RPB area was found for P, followed by U and Y. In particular, the mean score for P was 61.1±11.4%, while U exhibited 36.8±4.5%. In contrast, specimens treated with Y showed a mean RPB area of 5.8±5.1%. Thus, highest percent CIS areas was assessed for Y, followed by U and P. In particular, the mean score for Y was 94.2±5.1%, while U gave 63.2±4.5%. In contrast, specimens treated with P showed a mean CIS area of 38.9±11.2% (Fig. 4). Differences between the groups were statistically significant (P<0.001, respectively). The mean amount of time that was needed for implant surface instrumentation is presented in Fig. 6. An inability to remove RPB areas was felt by the operator after 5.6±1.2 min in the Y group, after 2.4±0.5 min in the U group, and after 2.3±0.5 min in the P group. The differences between Y on the one hand, and U and P on the other hand in terms of treatment time were statistically significant (P<0.001, respectively).

Cell viability

During the experimental period, there were no signs of any bacterial or fungal contamination of the well chambers. The mitochondrial activity of SAOS2 osteoblasts expressed as luminescent output (counts/s) is presented in Fig. 7. Highest cell viability was measured for C, followed by U, Y and P. In particular, measurement of luminescent output yielded the following mean scores: C (1.528.636±188.371) > U (831.594±370.228; P<0.001) > Y (678.250±367.902; n.s.) > P (144.105±120.961; P<0.001) (Fig. 7).

Discussion

The results of the present study indicate that all treatment procedures result in a significant decrease in plaque biofilm area grown on SLA titanium implants. When interpreting the present results, it must also be noted that, from a time-dependent viewpoint, Y was more effective at removing plaque biofilms than U or P. The design of the intraoral splint used to collect an in vivo biofilm in the present investigation followed, in general, the pattern described in previous studies [5, 14]. However, some modifications were made to the splint design in order to allow better and more comfortable plaque growth. The inserted titanium discs were turned towards the palate to avoid any disturbance by the tongue, but some space was permitted to provide a nutritious aqueous environment. Generally, all titanium discs were covered by a homogenous plaque biofilm after 24 h in situ, highlighting that rough SLA surface facilitates early plaque formation. This is in agreement with the results of previous studies, which have shown that early in vivo plaque accumulation is directly related to the surface roughness of the different test specimens (enamel, dentine, prosthetic materials, rough titanium surfaces), and that a homogenous and mature plaque biofilm is exhibited after 24 h [32, 41]. The results of the present investigation corroborate, to a certain extent, previous findings from a case report study evaluating the effectiveness of Y at removing subgingival debris from titanium implants under clinical conditions [36]. This investigation was conducted on eight implants (SLA and titanium plasma flamed-TPS) of two patients, considered for explantation due to severe bone loss and inflammation. Immediately before explantation, six implants were instrumented subgingivally with Y (12.7 J/cm^2), while two implants served as a control. In comparison to the untreated control specimens, nonsurgical instrumentation of titanium implants with this type of laser resulted in effective removal of subgingival debris without any thermal damage. However, all samples of the test group revealed some residual debris [36]. To the best of our knowledge, these are the first data reporting on the effectiveness of U used with a PEEK probe and the HA polishing fluid or P for the removal of plaque biofilms grown on SLA titanium implants. Although morphometrical analysis revealed significantly higher percentages of RPB areas on all ultrasonically-treated titanium discs than for those in the Y group, its effectiveness seemed to be significantly higher than for P. In this context, it is important to point to the results of a recent in vitro study which have shown that treatment of periodontally diseased root surfaces with U using a straight metal probe and the HA polishing fluid

resulted in less effective removal of subgingival dental calculus, but preservation of more tooth substance than a conventional ultrasonic system or hand instruments [15]. There might be several explanations for this discrepancy. First of all, it is important to realize that a supragingival plaque biofilm, collected artificially after a period of 24 h, is non-mineralized, whereas subgingival dental calculus is defined as mineralized dental plaque that is permeated with crystals of various calcium phosphates [35]. Furthermore, it has been reported that subgingivally, rough titanium surfaces harbour 25 times more bacteria, with increased proportions of Gram-negative anaerobic and facultative anaerobic species [3, 30, 31]. Therefore, the removal of subgingival plaque biofilms from rough titanium implants appears to be more difficult to achieve than the removal of non-mineralized supragingival plaque biofilms. On the other hand, the polishing fluid used contained HA, which also may have ameliorated the effectiveness of U, especially on rough titanium surfaces. Further studies are needed to clarify this issue. When interpreting the results of the present study, it was also noted that human SAOS2 osteoblasts responded differently to implant surfaces treated with either Y, U or P, as evaluated by means of cell viability measurement. Highest cell viability was measured for U, followed by Y, and P exhibiting the least mitochondrial activity. However, it must also be noted that the viability of SAOS2 osteoblasts in all test groups was significantly lower than for unworn and untreated control specimens. There might be several explanations for the present findings. First, it must be emphasized that mitochondrial activity was measured using an ATP-based luminescent cell viability assay, which has been reported to be more sensitive than other methods [1, 9, 21, 29]. 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 the culture [10]. To the best of our knowledge, there are no other data investigating the effects of U, used with a PEEK fibre tip, on the biocompatibility of SLA titanium implants. However, in this context, it is important to point to the results of a recent study evaluating the effects of Y and U, used with a carbon fibre tip, on the attachment and proliferation of human SAOS2 osteoblasts on differently structured titanium implants [37]. It was reported that all of the titanium discs treated with Y demonstrated almost the same cell density per mm^2 as untreated control surfaces. However, there was a significant decrease in the number of cells that attached to implant surfaces treated with U. The SEM examination showed no visible differences between lased and C titanium surfaces. In contrast, all surfaces treated with U showed debris from the used carbon fibres. The authors concluded that one possible explanation for the reduced cell numbers in the U-treated group was a cytotoxic effect from the debris of the used carbon fibres [37]. One possible explanation for the reduced cell numbers in the Y, U and P groups of the present study may be due to residual plaque biofilm areas which might have influenced the viability of SAOS2 cells. Indeed, recent studies have demonstrated that plaque biofilms may alter the surface characteristics of titanium surfaces. It was presumed that bacterial contamination of a titanium surface may affect its dioxide layer, resulting in a lower surface energy and therefore reduced tissue integration [7, 40]. Another possible explanation for the reduced biocompatibility of the titanium discs may be surface damage caused by Y, U or P. In this context, it must be pointed out that one limitation of the present study was the lack of a method to monitor morphological changes following treatment in all test groups. However, the results from recent studies have shown that Y, used with a cone-shaped glass fibre tip, did not cause any thermal surface damages on differently structured titanium implants at an energy density of 12.7 J/cm^2 [36, 37]. Similar results were also reported for P, since plastic instruments hardly altered the titanium implant surface following instrumentation [13, 22]. Furthermore, the results of the present study revealed an inverse relation between remaining plaque and cell viability between Y and U surfaces. This discrepancy might be explained by the fact that the polishing fluid used during ultrasonic instrumentation contains hydroxyapatite particles, which are basic in nature and may produce surface pH changes more favourable to cellular attachment. However, further studies are needed in order to determine whether such chemical interactions can enhance cell attachment.

Conclusion

Within the limits of the present study, it may be concluded that method Y (an Er:YAG laser at 100 mJ/pulse, 10 Hz) appears to be most suitable technique for the removal of supragingival early plaque biofilms grown on SLA titanium implants, and (2) that all treatment procedures failed to restore the biocompatibility of previously contaminated SLA titanium surfaces.

Acknowledgements This study was supported by a grant of the "Arbeitsgemeinschaft für Kieferchirurgie innerhalb der Deutschen Gesellschaft für Zahn-, Mund- und Kieferheilkunde".

References

1. Ahmad M, McCarthy MB, Gronowicz G (1999) An in vitro model for mineralization of human osteoblast-like cells on implant materials. Biomaterials 20:211–220
2. Albrektsson T, Isidor F (1994) Consensus report of session IV. In: Lang NP Karring T (eds) Proceedings of the First European Workshop on Periodontology. Quintessence, London, pp 365–369
3. Alcoforado GA, Rams TE, Feik D, Slots J (1991) Microbial aspects of failing osseointegrated dental implants in humans. J Parodontol 10:11–18
4. Augthun M, Tinschert J, Huber A (1998) In vitro studies on the effect of cleaning methods on different implant surfaces. J Periodontol 69:857–864
5. Auschill TM, Arweiler NB, Netuschil L, Brecx M, Reich E, Sculean A, Artweiler NB (2001) Spatial distribution of vital and dead microorganisms in dental biofilms. Arch Oral Biol 46:471–476

6. Bach G, Neckel C, Mall C, Krekeler G (2000) Conventional versus laser-assisted therapy of periimplantitis: a five-year comparative study. Implant Dent 9:247–251
7. Baier RE, Meyer AE (1988) Implant surface preparation. Int J Oral Maxillofac Implants 3:9–20
8. 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 5:31–38
9. Cree IA, Pazzagli M, Mini E, Mazzei T, Hunter EM, Sutherland LA, Pinzani P, Gerli A, Andreotti PE (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 6:398–404
10. 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 160:81–88
11. 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 30:511–518
12. 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 7:320–328
13. 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 61:485–490
14. Hahn R, Netuschil L, Löst C (1992) Initiale Plaquebesiedlung auf keramischen Restaurationsmaterialien. Dtsch Zahnärztl Z 47:330–334
15. Hartschen VJ, Frentzen M (2002) Effects of the ultrasonic Vector system compared to conventional ultrasonic and hand instrumentation (in German). Parodontologie 13:133–142
16. 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 23:299–309
17. Kreisler M, Al Haj H, Götz H, Duschner H, d'Hoedt B (2002) Effect of simulated CO(2) and GaAlAs laser surface decontamination on temperature changes in Ti-plasma sprayed dental implants. Lasers Surg Med 30:233–239
18. Kreisler M, Götz H, Duschner H (2002) 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 17:202–211
19. Kreisler M, Kohnen W, Marinello C, Götz H, Duschner H, Jansen B, d'Hoedt B (2002) Bactericidal effect of the Er:YAG laser on dental implant surfaces: an in vitro study. J Periodontol 73:1292–1298
20. Löe H (1967) The gingival index, the plaque index and the retention index systems. J Periodontol 38(Suppl):610–616
21. 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 23:273–276
22. Mengel R, Buns CE, Mengel C, Flores-de-Jacoby L (1998) An in vitro study of the treatment of implant surfaces with different instruments. Int J Oral Maxillofac Implants 13:91–96
23. Mombelli A, Buser D, Lang NP (1988) Colonization of osseointegrated titanium implants in edentulous patients. Early results. Oral Microbiol Immunol 3:113–120
24. Mombelli A, Lang NP (1992) Antimicrobial treatment of periimplant infections. Clin Oral Implants Res 3:162–168
25. Mombelli A, Lang NP (1994) Microbial aspects of implant dentistry. Periodontol 2000 4:74–80
26. Moritz A, Schoop U, Goharkhay K, Schauer P, Doertbudak O, Wernisch J, Sperr W (1998) Treatment of periodontal pockets with a diode laser. Lasers Surg Med 22:302–311
27. Oyster DK, Parker WB, Gher ME (1995) CO_2 lasers and temperature changes of titanium implants. J Periodontol 66:1017–1024
28. Parham PL Jr, Cobb CM, French AA, Love JW, Drisko CL, Killoy WJ (1989) Effects of an air–powder abrasive system on plasma-sprayed titanium implant surfaces: an in vitro evaluation. J Oral Implantol 15:78–86
29. 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 10:29–34
30. Quirynen M, van der Mei HC, Bollen CM, Schotte A, Marechal M, Doornbusch GI, Naert I, Busscher HJ, van Steenberghe D (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 72:1304–1309
31. Rams TE, Roberts TW, Tatum H Jr, Keyes PH (1984) The subgingival microbial flora associated with human dental implants. J Prosthet Dent 51:529–534
32. Rimondini L, Fare S, Brambilla E, Felloni A, Consonni C, Brossa F, Carrassi A (1997) The effect of surface roughness on early in vivo plaque colonization on titanium. J Periodontol 68:556–562
33. 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 71:810–815
34. 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 8:427–433
35. Schroeder HE (1965) Crystal morphology and gross structures of mineralizing plaque and of calculus. Helv Odontol Acta 35:73–86
36. Schwarz F, Rothamel D, Becker J (2003) Influence of an Er:YAG laser on the surface structure of titanium implants. Schweiz Monatsschr Zahnmed 113:660–671
37. Schwarz F, Rothamel D, Sculean A, Georg T, Scherbaum W, Becker J (2003) 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 14:784–792
38. Schwarz F, Sculean A, Berakdar M, Szathmari L, Georg T, Becker J (2003) 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 32:359–366
39. Schwarz F, Sculean A, Rothamel D, Schwenzer K, Georg T, Becker J (2005) Clinical evaluation of an Er:YAG laser for nonsurgical treatment of peri-implantitis: a pilot study. Clin Oral Implants Res 16(1):44–52
40. 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 4:23–27
41. 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 2:38–46
42. Tucker D, Cobb CM, Rapley JW, Killoy WJ (1996) Morphologic changes following in vitro CO_2 laser treatment of calculus-ladened root surfaces. Lasers Surg Med 18:150–156
43. 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 46:64–71