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In Vitro-Activity of Er:YAG Laser in Comparison with other Treatment Modalities on Biofilm Ablation from Implant and Tooth Surfaces

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Abstract

Background and Aim

Bacterial biofilms play a major role in the etiology of periodontal and peri-implant diseases. The aim of the study was to evaluate the removal of bacterial biofilms and attachment of epithelial cells (EC), gingival fibroblasts (GF) and osteoblast-like cells (OC) to dentin and titanium surfaces after Er:YAG laser (Er:YAG) in comparison with other treatment methods.

Material and Methods

Multi-species bacterial biofilms were grown on standardized dentin and titanium specimens with a sand-blasted and acid etched (SLA) surface for 3.5 d. Thereafter, the specimens were placed into artificially-created pockets. The following methods for biofilm removal were used: 1) Gracey (dentin) or titanium curettes (CUR), 2) Er:YAG, 3) photodynamic therapy (PDT) and 4) CUR with adjunctive PDT (CUR/PDT). Colony forming units (CFUs) of the remaining biofilms and attachment of EC, GF and OC were determined. Statistical analysis was performed by means of ANOVA with post-hoc LSD.

Results

All treatment methods decreased statistically significantly ($p<0.001$) total CFUs in biofilms compared with untreated dentin and titanium surfaces respectively. On dentin, Er:YAG was equally efficient as CUR and PDT but inferior to CUR/PDT ($p = 0.005$). On titanium, surfaces, the use of Er:YAG resulted in statistically significantly superior biofilm removal compared to the 3 other treatments (each $p<0.001$). Counts of attached EC, GF and OC were the lowest on untreated contaminated dentin and titanium surfaces each. After CUR/PDT higher EC counts were found on dentin ($p = 0.006$). On titanium, all decontamination methods statistically significantly increased ($p<0.001$) the counts of attached EC without differences between groups. Statistically significantly higher counts of GF ($p = 0.024$) and OC ($p<0.001$) were observed after Er:YAG decontamination compared with untreated surfaces.

Conclusion

Ablation of subgingival biofilms and in particular decontamination of titanium implant surfaces with an Er:YAG laser seem to be a promising approach and warrants further investigations.

Introduction

Lasers have been introduced in dentistry in the 60ies of last century. In one of the first reports a ruby laser with an energy density of about 9'000 J / cm^2 was applied for destruction of carious lesions in in-vitro experiments [1]. Meanwhile, lasers were introduced in nearly all fields of dentistry including endodontics, periodontology, implantology, oral surgery, orthodontics and cariology [2]. The term "LASER" stands for "Light Amplification by Stimulated Emission of Radiation". The principle is that electrons being normally in a low energy state are transferred to a high energy state; when moving back to the low energy state the absorbed energy is released. Lasers used in dentistry are differentiated between low-intensity (*e.g.* diode lasers) and high-intensity lasers (*e.g.* Erbium-Doped: Yttrium, Aluminum, Garnet (Er:YAG) laser). In periodontal therapy low-intensity lasers are discussed to improve wound healing and combined with photosensitizer (photodynamic therapy) they can act exert antimicrobial activity [3].

Er:YAG lasers with a wavelength of 2'940 nm can be used both for soft and hard-tissue surgery as well as for removal of subgingival calculus from periodontal pockets. Its laser light is absorbed in superficial layers and thus Er:YAG laser does not penetrate deeply into the tissues. The efficacy in soft and hard tissue is explained as evaporation by photo-thermal interactions [4]. Er:YAG laser was proven to act antimicrobial. When applying to microbes spread on agar plates a clear inhibition zone was found both for microbes being associated with endodontic (e.g. *Candida albicans*, *Enterococcus faecalis*) [5] and periodontal infections (e.g. *Porphyromonas gingivalis*) [6].

Treatment of periodontal and peri-implant diseases includes various anti-infectious regimens. The primary goal is to remove hard and soft bacterial deposits leading to a smooth and biocompatible surface in order to minimize further bacterial adhesion and to facilitate host cell re-attachment. In the treatment of periodontal disease, scaling and root planing (SRP), which includes mechanical removal of biofilms, is an effective causative method for infection control [7]. On the other hand, in peri-implant diseases, mechanical non-surgical therapy alone may be effective in the management mucositis but not of peri-implantitis lesions [8].

Er:YAG lasers have been often discussed as a treatment options for removal of subgingival and peri-implant biofilms; available evidence suggests that subgingival and submucosal debridement with Er:YAG laser treatment may reduce periodontal and peri-implant mucosal inflammation [9,10,11]. In periodontitis treatment outcomes, a recent systematic review indicated that Er:YAG laser was as effective as scaling and root planing 3 months after therapy [12]. In peri-implantitis treatment, a meta-analysis including data of four randomized clinical trials found short-term benefits in terms of probing depth reduction after Er:YAG but a general superiority to mechanical debridement was not observed [13].

Subgingival and peri-implant biofilms are communities consisting of numerous bacteria. Organisms strongly being associated with periodontal disease include *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* [14,15]. Bacteria associated with peri-implant lesions are at least partially the same as in periodontitis. Black pigmented *Prevotella* species, *A. actinomycetemcomitans*, *P. gingivalis* were assessed in

higher quantities from peri-implantitis lesions than from healthy controls [16,17]. In patients having a history of treated gingivitis or periodontitis, periodontopathogens (*P. gingivalis*, *T. forsythia*, *T. denticola*, *Fusobacterium nucleatum*, *Prevotella intermedia*) were already detectible 30 minutes after implant placement [18] and one week following abutment connection [19]. In a retrospective cross-sectional study analyzing implants and the adjacent teeth 10 years after placement, more *T. forsythia*, *Parvimonas micra*, *F. nucleatum / necrophorum* and *Campylobacter rectus* were found at implants than at teeth [20]. The counts correlated positively for all these species at implants and teeth and a high correlation was observed for *P. gingivalis* (R = 0.503) [20]. Taken together, the available data from the literature clearly suggest, that at present, there is a stringent need for developing novel approaches for predictable decontamination of implant surfaces in order to determine the best suitable method for biofilm removal and creation of a surface favoring adhesion of epithelial cells, gingival fibroblasts, and osteoblast-like cells.

Therefore, the aims of the present in-vitro study were: (i) to evaluate the activity of Er:YAG laser in killing selected planktonic microorganisms and to compare its efficacy with that of other treatment modalities (hand instrumentation with using curettes, photodynamic therapy, hand instrumentation combined with photodynamic therapy) on the destruction and removal of a 12-species biofilm on dentin and titanium surfaces and (ii) to evaluate and compare the adhesion of epithelial cells, gingival fibroblasts, and osteoblast-like cells on the dentin and titanium surfaces following destruction and removal of a 12-species biofilm following Er:YAG laser application and other treatment modalities.

Materials and Methods

Microorganisms

The following bacterial strains were included in the study: *Streptococcus gordonii* ATCC 10558, *Actinomyces naeslundii* ATCC 12104, *F. nucleatum* ATCC 25586, *Campylobacter rectus* ATCC 33238, *Filifactor alocis* ATCC 35896, *Eikenella corrodens* ATCC 23834, *P. intermedia* ATCC 25611, *P. micra* ATCC 33270, *P. gingivalis* ATCC 33277, *T. forsythia* ATCC 43037, *T. denticola* ATCC 35405 and *A. actinomycetemcomitans* Y4.

Before an experiment, all strains (except for *T. denticola* ATCC 35405) were precultivated on Schaedler agar plates (Oxoid, Basingstoke, UK) with 5% sheep blood in an anaerobic atmosphere or with 5% CO_2 (*A. actinomycetemcomitans* Y4 and *S. gordonii* ATCC 10558). *T. denticola* was maintained in modified mycoplasma broth (BD, Franklin Lake, NJ) added by 1 mg/ml glucose, 400 μg/ml niacinamide, 150 μg/ml spermine tetrahydrochloride, 20 μg/ml Na isobutyrate enriched with 1 g/ml cysteine and 5 μg/ml cocarboxylase in anaerobic conditions.

Antimicrobial activity on planktonic bacteria

A defined inoculum of microorganisms (10^6 and 10^3 in 100 μl RPMI 1640 without phenol red) was prepared and pipetted on glass slides which were transferred to a 50 ml tube. Thereafter, Er:YAG laser (Erwin AdvErl, J. Morita) was applied with irradiation powers 30 mJ, 50 mJ and 70 mJ (each on panel)-20 pps for 10 s, 20 s and 3 x 20 s (equivalent to 0.6 W, 1 W, 1.4 W). The numbers of colony forming units (cfu) were determined after addition of NaCl 0.9% solution, preparing a 10-fold dilution series and plating of each 100 μl on agar-plates. In these experiments selected single species were tested in independent replicates.

Test specimens for biofilm formation

Dentin specimens were prepared from porcine teeth being a by-pass product in slaughterhouse. Teeth were removed from the jaws and placed in chloramine solution for disinfection.

The crowns of the teeth were removed and dentin slices of the buccal side of the roots were cut with diamond disks (~ 6 × 12 mm) with a thickness of ~3 mm. The surface properties of the buccal side of the dentin specimens were standardized by grinding with silicon carbide papers of #2400 grit size, corresponding to an abrasive particle size of 6.5 μm. Finally the dentin slices were fixed on plastic disks as described recently [21].

Titanium specimes with a sandblasted and acid-etched (SLA) surface (Institut Straumann AG, Basel, Switzerland) were fixed on plastic specimens similarly as described above.

Biofilm formation

The dentin and titanium specimens were covered with a 5% chicken serum albumin solution for 1h and thereafter placed in tubes. Suspensions of 12 bacterial strains were mixed with nutrient broth and transferred to the tubes. After incubation in anaerobic conditions for 72 h, two thirds of the medium were exchanged and *P. gingivalis* ATCC 33277, *T. forsythia* ATCC 43037 and *T. denticola* ATCC 35406 were be added again. After final incubation of 48 h, the medium was removed and the treatment methods were applied.

Methods for biofilm removal

Plastic specimens with the dentin or titanium disks were transferred in the pocket model as described previously [21]. In this model, instrumentation was performed obliquely to the dentin and titanium surfaces thus mimicking as close as possible the clinical situation. Standardized test specimens were used allowing a comparison between the different treatment methods and in addition between different surfaces like dentine and titanium. However, it should be noted, that the curved shape of teeth, a potential coating with calculus as well as the niches and the different surfaces of a dental implant were not considered.

In biofilm experiments irradiation power was 50 mJ (on panel)-20 Hz per pulse for titanium and 70 mJ (on panel)-20 Hz per pulse for dentin. The energy was chosen based on the manufacturer's recommendation; higher energy was shown to damage the titanium surface [22]

For all Er:YAG laser applications, the PS600 T tip was used in the respective handpiece. The laser was used in an oblique angle for 15 s with concomitant water spray irrigation (5 ml / min) in contact with the surface of the dentine and in non-contact with the surface of titanium disk.

Three positive control treatments were used. In case of dentin specimens, Gracey curettes (CUR) made of stainless steel (Deppeler SA, Rolle, Switzerland) while in case of titanium plates, the instrumentation was performed by means of titanium curettes (Deppeler SA, Rolle, Switzerland). Specimens were instrumented from apically to coronally by means of 20 strokes using both sites of the curettes. Each side of the curette was used for five specimens and then replaced by a new curette. Before instrumentation, after every 10th stroke and at the end of instrumentation, the dentin and titanium surfaces in the pockets were rinsed with 2.5 ml of 0.9% w/v NaCl. The second positive control was photodynamic therapy (PDT) applying 25 μl of phenothiazine chloride (HELBO® Blue Photosensitizer; Photodynamic Systems GmBH, Wels, Austria) as photosensitizer for 3 min combined with 2 × 10 s of a hand-held diode laser (HELBO® 3D Pocket, Photodynamic Systems GmbH) with a wavelength of 660 nm and a power density of 100 mW according to the manufacturer's instruction before final rinsing with 2.5 ml of 0.9% w/v NaCl. The third positive control combined CUR (10 strokes) and PDT as described above (CUR/PDT).

Untreated contaminated specimens served as negative controls (con). Additionally, in cell adhesion assays, non-contaminated specimens were included as controls (con cells).

Influence of treatment methods on bacteria

Immediately after treatments, biofilm samples were collected from the surface and suspended in 0.9% w/v NaCl solution. After making a serial dilution each 25 μl was spread on Schaedler agar plates, incubated in anaerobic conditions and the total colony forming units (cfu) counts incl. black-pigmented were assessed. Further, the loads of *P. gingivalis*, *T. forsythia*, *P. micra* were determined by using real-time PCR [23] and those of *F. nucleatum* by counting relevant CFU.

Influence of biofilm removal on adhesion of epithelial cells, gingival fibroblasts and osteoblast-like cells

Epithelial TIGK cells donated by R Lamont, University of Louisville, USA were used. Cells were maintained in Keratinocyte growth medium (Ruwag Handels AG, Bettlach, Switzerland). Human alveolar osteoblasts (HAO) as well as human gingival fibroblasts were obtained from periodontally healthy patients during surgery (extraction of teeth for orthodontic reasons) and processed as described recently [24]. Cells were anonymously collected from patients during regular periodontal or implant treatment following written informed consent. This procedure is approved by the Ethics Committee of the University of Bern. The cultivation medium was DMEM (DMEM, Invitrogen, Carlsbad, CA) 0% of fetal bovine serum (FBS, Invitrogen). For experiments, both cell types were used from passages 4–6.

After applying the different treatment methods and removing the test specimens from the artificial pockets, the dentin and titanium disks were carefully removed from the plastic specimens. Before seeded into cell-culture-well-plates with the respective cells disks were exposed to UV to avoid bacterial overgrowth in the cell culture media [21]. About 5×10^4 cells per well were added. Cells were incubated with 5% CO_2 for 72 h. Afterwards, cells were fixed and stained with DAPI (Roche Diagnostics GmbH, Mannheim, Germany) for cells being exposed to titanium and with Pappenheim (Hemacolor®, Merck Millipore, Darmstadt, Germany) for those being exposed to dentin. Ten fields of 1 mm^2 each were counted. Fields were equally distributed from the whole slide.

All biofilm experiments were conducted in independent quadruplicates in two series.

Statistical analysis

Data used in statistical analysis are presented as mean and standard deviation (SD). In case of bacterial counts $\log_{10}$ values are reported. Data were compared using a one-way analysis of variance (ANOVA) with post-hoc comparisons of groups using LSD corrections. A *p*-value of 0.05 was considered to be statistically significant. SPSS software (version 22.0) was used for statistical analysis.

Results

Planktonic bacteria

As no dependence of the irradiation power was visible in first experiments, only the highest power (70 mJ) and longest time (3 times 20 s) were used in the follow-up experiments. However, differences to untreated control did not exceed 0.15 $\log_{10}$ CFU (Fig 1).

Biofilm removal from dentin disks

All treatment procedures reduced statistically significantly (each $p<0.01$) the total bacterial counts. Er:YAG reduced the bacterial counts by 2.44 $\log_{10}$ with no significant difference to

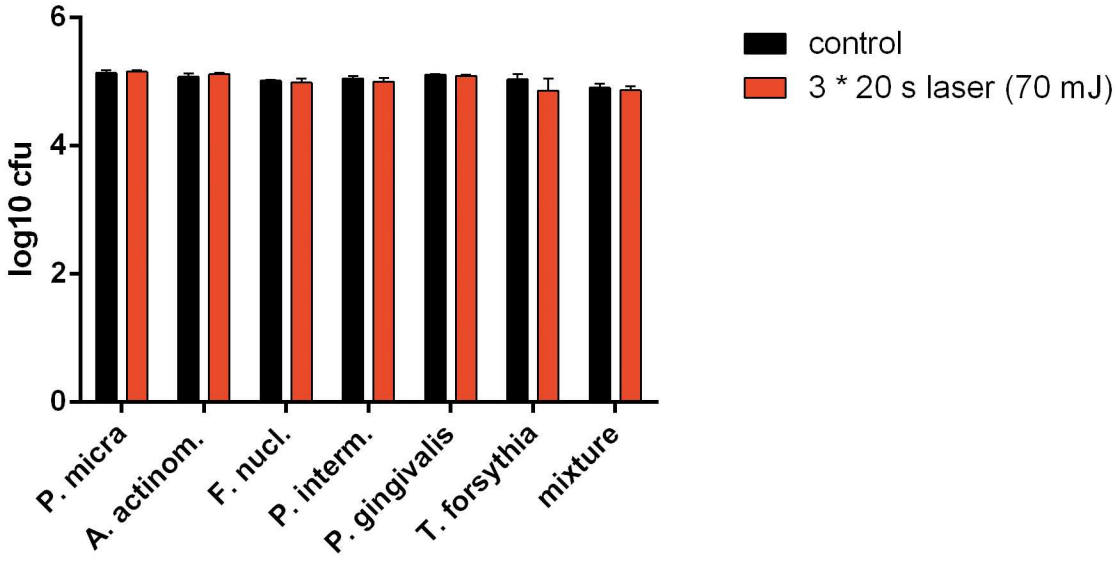

Fig 1. Killing of planktonic bacteria. Killing of selected bacterial species and a 12-species mixture after 3*20 s of laser irradiation with a power of 70 mJ.

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CUR (reduction 1.71 $\log_{10}$). The lowest values of remained bacteria (reduction by 4.01 $\log_{10}$) were seen for CUR/PDT each being significantly different ($p<0.01$) to all other treatments incl. Er:YAG ($p = 0.005$). The analyzed single species were also statistically significantly reduced in comparison with the untreated control. Counts of *P. gingivalis* ($p = 0.008$), *T. forsythia* ($p = 0.026$) and *F. nucleatum* ($p = 0.002$) were statistically significantly lower after CUR/PDT than after applying PDT alone (Fig 2).

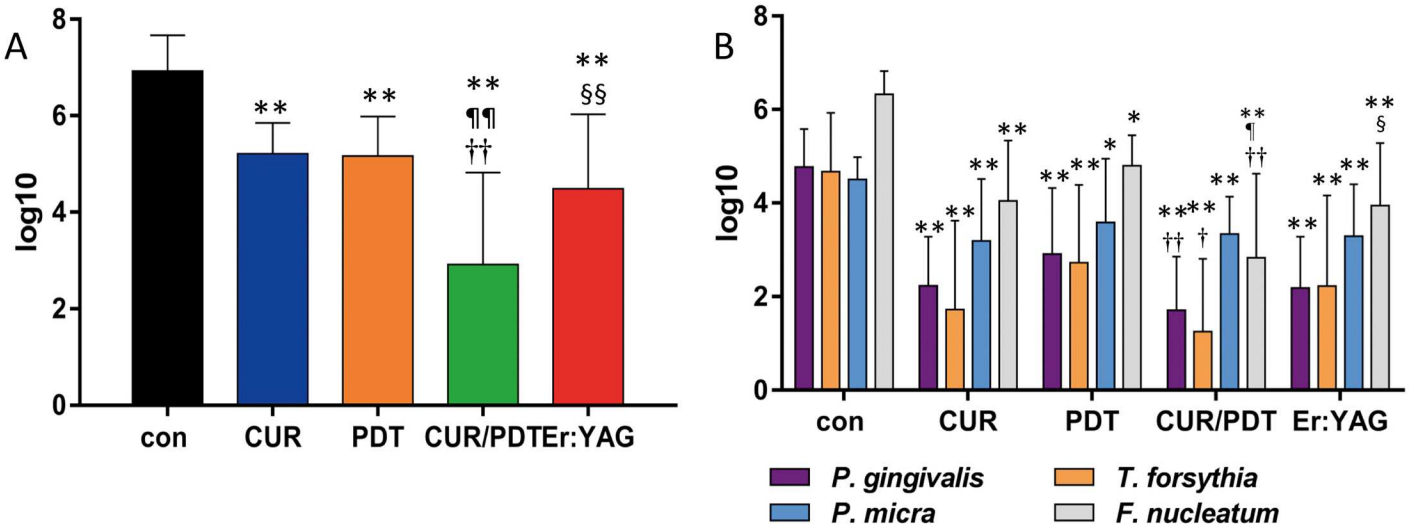

Fig 2. A-B. Biofilm removal from dentine disks. Remains of biofilm after exposing to Gracey curette (CUR), photodynamic therapy (PDT), CUR combined with PDT (CUR/PDT) and Er:YAG laser irradiation (Er:YAG) Presented are total counts (cfu; A) and counts for selected species (B) * $p<0.05$; ** $p<0.01$ compared with control (con) ¶ $p<0.05$; ¶¶ $p<0.01$ compared with CUR † $p<0.05$; †† $p<0.01$ compared with PDT § $p<0.05$; §§ $p<0.01$ compared with CUR/PDT.

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Adhesion of gingival epithelial cells and PDL fibroblasts after biofilm removal to dentin disks

Attachment of gingival epithelial cells dropped down close to zero if the biofilm was not removed (difference to con cells $p<0.001$). After treatment of the surface, the numbers increased but the difference to the untreated biofilm-exposed sample was only statistically significant in the treatment performed with CUR/PDT ($p = 0.006$). Except for CUR/PDT, following all other treatments, the significance to con cells remained (each $p<0.05$).

The number of PDL fibroblasts dropped also down after exposing them to biofilms ($p<0.001$). No clear increase in their counts was seen after any treatment and the difference to con cells remained (each $p<0.001$) (Fig 3).

Biofilm removal on titanium disks. All treatment procedures reduced statistically significantly (each $p<0.01$) the total bacterial counts. The lowest values of remaining bacteria (reduction by 4.45 $\log_{10}$) were seen for Er:YAG laser each being statistically significantly different ($p<0.01$) to PDT and CUR (total counts). Moreover, counts of *P. gingivalis* and *T. forsythia* were less numerous than following PDT alone and CUR (Fig 4).

Adhesion of gingival epithelial cells, gingival fibroblasts and osteoblast-like cells after biofilm removal to titanium disks. Attachment of gingival epithelial cells dropped down if the biofilm was not removed (difference to con cells $p<0.001$). Following any of the used treatments, the numbers increased statistically significantly ($p<0.01$ except for CUR/PDT ($p = 0.016$)). A statistical significant difference to cell adherence to titanium surfaces without bacterial biofilm exposure was observed (each $p<0.001$).

The number of PDL fibroblasts decreased after exposing them to biofilms ($p = 0.048$). The difference was also statistically significant when PDT was applied for biofilm removal ($p = 0.016$). A clear increase in their counts was seen following treatment with Er:YAG and the difference was statistically significant compared to con ($p = 0.024$) and PDT ($p = 0.007$).

The number of the attached osteoblasts did not appear to be affected by exposing titanium to bacterial biofilm. However, following removal of biofilm with Er:YAG laser, the osteoblasts adhered in a statistically significant higher number (each $p<0.01$) compared with any of the controls or other treatment groups (Fig 5).

Discussion

The present *in-vitro* study has evaluated the application of Er:YAG laser in comparison with hand instruments and photodynamic therapy in ablation of periodontal and peri-implant biofilms.

The first series of experiments focused on the activity of Er:YAG laser against planktonic bacteria. Up to now, the bactericidal activity of Er:YAG laser on oral bacteria was only very rarely investigated. The missing bactericidal activity on planktonic bacteria is in contrast with a few previous studies reporting bactericidal activity [6,25,26]. In our study, the bacteria were placed on glass slides before laser irradiation. Since Er:YAG laser can only be used in conjunction with water cooling (i.e. irrigation), it cannot be completely ruled out that, at least some bacteria were swept off fast and thus were not directly exposed to the laser beam. However, when Er:YAG laser was used without water cooling (data not shown) no bactericidal activity was observed. In some other studies, Er:YAG laser was applied to bacteria on agar plates [6,25], and therefore an absorption of the laser by the agar and evaporation of the agar might have also influenced the results. In one study where microorganisms were spread on agar or were incorporated in the agar, only superficially located microorganisms and not those located in deeper layers, were affected by Er:YAG laser [5]. This finding suggests that the reduction of bacterial counts in biofilms is rather ablative than bactericidal.

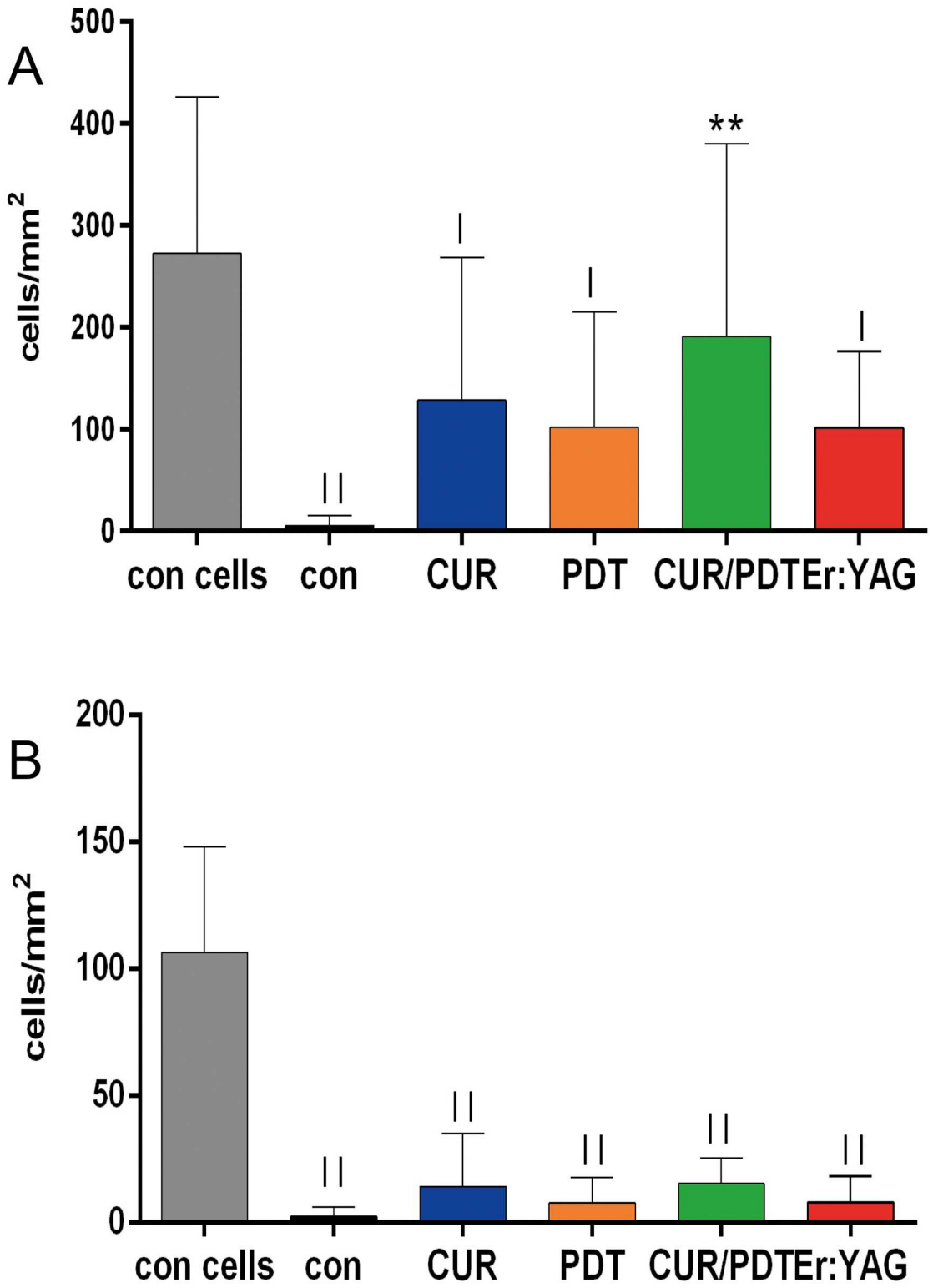

Fig 3. A-B. Adhesion of cells after biofilm removal from dentine disks. Adhesion of gingival epithelial cells (A) and PDL fibroblasts (B) after biofilm removal by applying Gracey curette (CUR), photodynamic therapy (PDT), CUR combined with PDT (CUR/PDT) and Er:YAG laser irradiation (Er:YAG) ** $p<0.01$ compared with control with bacteria (con) ^{I} $p<0.05$; ^{II} $p<0.01$ compared with control without bacteria (con cells).

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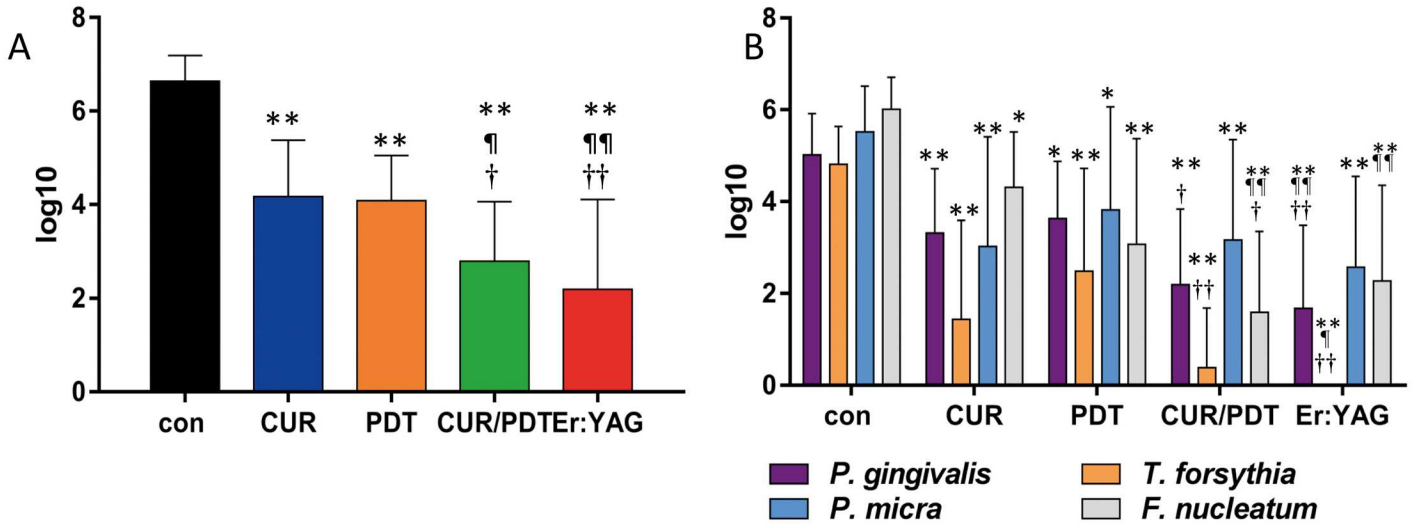

Fig 4. A-B. Biofilm removal from titanium disks. Remains of biofilm after applying titanium curette (CUR), photodynamic therapy (PDT), titanium curette combined with PDT (CUR/PDT) and Er:YAG laser irradiation (Er:YAG) Presented are total counts (cfu; A) and counts for selected species (B) * p<0.05; ** p<0.01 compared to control (con) ¶ p<0.05; ¶¶ p<0.01 compared to CUR † p<0.05; †† p<0.01 compared to PDT.

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In this study biofilms of comparable composition were established both on dentin and titanium disks, thus allowing direct comparison of the used methods. Well defined multi-species subgingival/periodontal biofilms on dentin and titanium disks were exposed to Er:YAG laser irradiation and compared with other treatment modalities including hand instrumentation alone. The reason to create an artificial "pocket" was to reproduce the clinical situation of narrow subgingival areas, thus enabling to perform the treatments in a way as close to the clinical situation as possible [21].

The only difference between the untreated biofilm on dentin and titanium surfaces was in the counts of *P. micra*, the species was found in higher numbers on titanium than on dentin disks (difference 1.07 $\log_{10}$; p = 0.005). In contrast to the missing bactericidal activity, ablation of biofilms from both dentin and titanium specimens by means Er:YAG laser was clearly observed. The differences to the untreated controls were 2.44 $\log_{10}$ for dentin and 4.45 $\log_{10}$ for titanium surfaces, respectively. On dentin, Er:YAG was as efficient as hand instrumentation alone. Reduction of viable counts within single-species biofilms (*P. gingivalis*, *F. nucleatum*, *A. naeslundii* and others) on hydroxyapatite disks by this laser type irradiating with 20–80 mJ / puls at 10 pps for 10 s has been reported previously [26] but superiority to other methods was not observed. This is in contrast to another study where extracted teeth were exposed to ultrasonication and Er:YAG laser [6]. In that study [6] at teeth partly covered with calculus, the total anaerobic counts in biofilm controls were only 3.71 $\log_{10}$ while comparison was made to ultrasonication. In the present study, ultrasonication was not included but in a recent study with similar methodology superiority of ultrasonication to hand instrumentation was reported [21].

Whereas no statistically significant differences were found compared to hand instrumentation on dentin, Er:YAG was most effective in reducing bacterial counts in biofilms on titanium. The difference between the two types of test specimens (dentin and titanium) was 2.02 $\log_{10}$ (p = 0.03). Er:YAG laser removed more efficiently early plaque than plastic curettes combined with chlorhexidine rinsing and ultrasonication from titanium disks embedded in acrylic splints worn by volunteers [27]. In vitro, Er:YAG decreased significantly viability of *Candida albicans* biofilm by using 100 mJ and an irradiation time of 80 s [28]. The high biofilm ablation

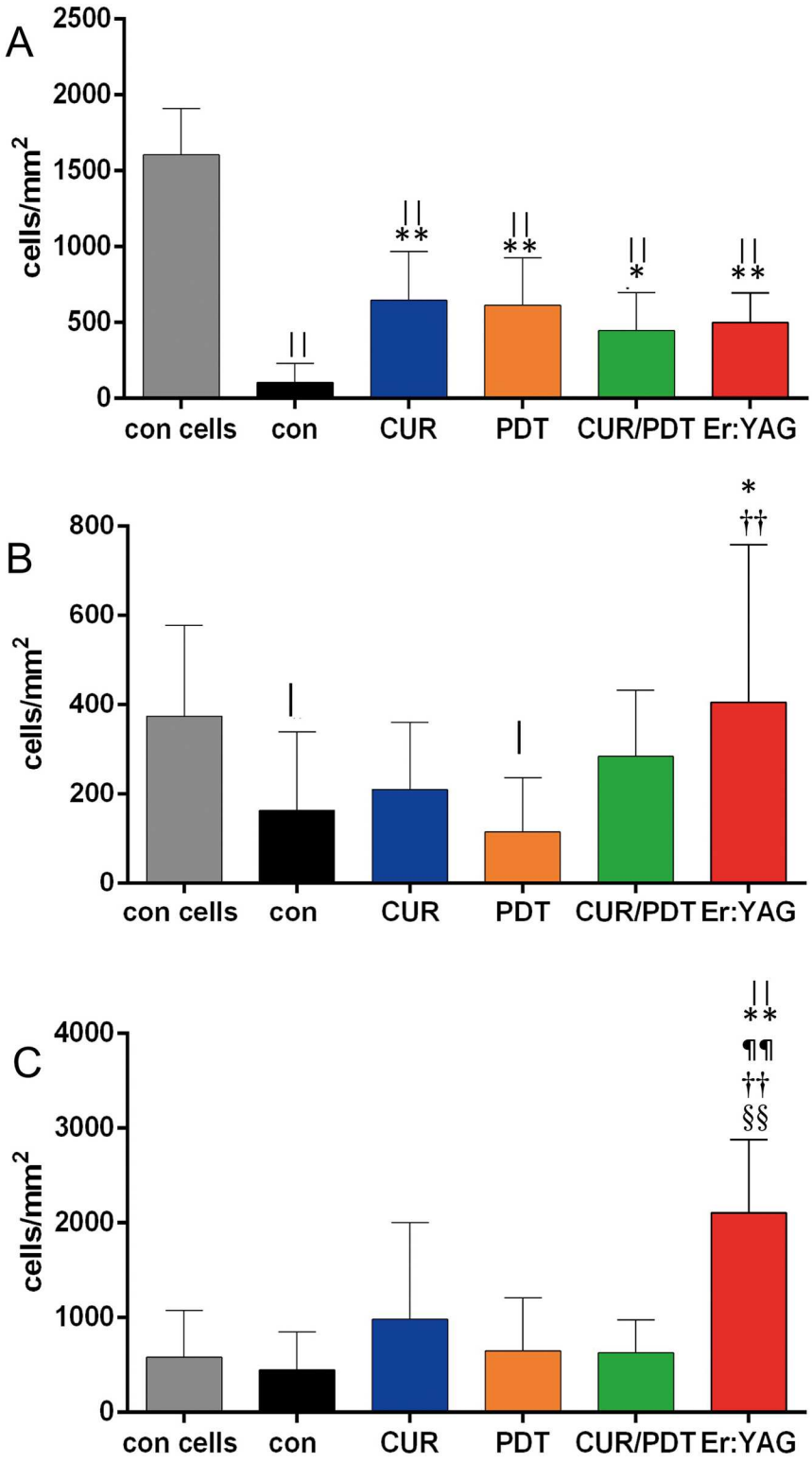

Fig 5. A-C. Adhesion of cells after biofilm removal from titanium disks. Adhesion of gingival epithelial cells (A), gingival fibroblasts (B) and osteoblast-like cells (C) before and after biofilm removal by after applying titanium curette (CUR), photodynamic therapy (PDT), titanium curette combined with PDT (CUR/PDT) and Er:YAG laser irradiation (Er:YAG) * p<0.05; ** p<0.01 compared with control with bacteria (con) $^{|}$ p<0.05; $^{||}$ p<0.01 compared with control without bacteria (con cells) ¶¶ p<0.01 compared with CUR †† p<0.01 compared with PDT §§ p<0.01 compared with CUR/PDT.

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by Er:YAG laser might be of particular importance, as in vivo the niches formed by the screw of the abutment cannot be reached by hand instruments.

Among the other tested methods, combination of CUR/PDT with a reduction of about 4 $\log_{10}$ was always more efficient than CUR or PDT alone, on both dentin and titanium. Several studies reported an additional improvement of clinical parameters when photodynamic therapy was applied additionally to scaling and root planing in the treatment of periodontitis [29,30]. Moreover, in the treatment of initial peri-implantitis, adjunctive photodynamic therapy was as effective as local antibiotic delivery in the reduction of sites with bleeding on probing [31].

Adhesion of host cells correlated inversely with the remained bacterial counts on the surface after various treatments. The lowest counts of epithelial cells were seen when non-disrupted biofilm was present, the highest without any previous bacterial contamination. Despite the fact that bacteria were inactivated by UV-light which is used to stop multiplication, virulence factors might still have been present. For example, *P. gingivalis* proteases can degrade a number of cell adhesion molecules and thus induce detachment and apoptosis of epithelial cells (Chen, Casiano *et al*. 2001). While any bacterial constituent clearly inhibited attachment of PDL fibroblasts to dentin, after treatment of titanium surfaces with Er:YAG laser, no differences in the attachment of gingival fibroblasts were observed compared to pristine test surfaces (i.e. a surfaces that have not been exposed to any bacterium). This is confirmatory to another in-vitro study where decontamination of a single-species biofilm of *P. gingivalis* by Er:YAG laser at pulse energy 60 mJ and frequency 10 pps in vitro did not interfere with growth of gingival fibroblasts thereafter [32]. Moreover, in beagle dogs the Er:YAG laser was applied to implants placed into the mandible without disturbing osseointegration [22].

An important finding of the present study was the high adhesion rate of osteoblast-like cells on the Er:YAG treated titanium surfaces which was even higher than that observed on pristine test specimens without any bacteria. This finding confirms previous reports on the good adhesion of a osteoblast-like cell line [27] and primary osteoblast-like cells [33] after decontamination of SLA titanium surface using Er:YAG laser.

Er:YAG laser has the highest absorption in water and a very high absorption in hydroxyapatite [34]. Therefore, it may be anticipated that the absorption of this laser on titanium is much lower and following, more energy can be absorbed by a biofilm that contains a high percentage of water. This phenomenon might in turn lead to biofilm ablation. Temperature in the closed surrounding of the titanium implant increase after 60 s Er:YAG irradiation 100 mJ 20 Hz by 10.5˚C with air/water [35]. Below 0.5 mm dentin rise in temperature is 3.86˚C when irradiating with 12.7 mJ / cm^2 20 Hz for 30 s with water cooling [36]. Micro-rough SLA surfaces at titanium are not damaged up to 140 mJ per pulse [37]. After Er:YAG irradiation with 100 mJ 10 Hz for 1 min to SLA surfaces, the surface roughness decreased while wettability increased and as a consequence, the subsequently adhered osteoblast-like cells exhibited a higher cell-viability [38]. On dentin Er:YAG laser increased wettability but also surface porosities [39].

Most studies which have compared microbiologically and clinically the outcomes following scaling and root planing with Er:YAG laser debridement in patients with chronic periodontitis failed to reveal any statistically significant differences in any of the analyzed parameters [40,41]. In only one study a greater reduction of *P. gingivalis* was observed at 12 months following the use of Er:YAG laser [42]. Nonsurgical treatment of peri-implantitis lesions at implants with a machined surface by means of Er:YAG laser revealed minor clinical and microbiological changes, [43] while in a pilot study, Er:YAG laser showed greater reduction in bleeding on probing when compared with the use of plastic curettes and chlorhexidine rinsing [44]. On the other hand, when Er:YAG laser was used during surgery no superior improvements compared with those obtained following the use of plastic curettes were found [45].

Conclusion

Within their limits, the present data indicate that: a) on dentin surfaces, Er:YAG laser appears to be equally effective as hand instrumentation for removing bacterial biofilms, b) the combination of CUR and PDT appears to be a suitable method to additionally decontaminate dentin surfaces, and c) on titanium surfaces, the use of Er:YAG laser yielded clear advantages compared to the other debridement modalities.

Supporting Information

S1 File. Supporting data to the Figs 1–5. The means and SD for Figs 1–5 incl. exact p-values are presented.
(DOCX)

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