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Influence of different treatment approaches on non-submerged and submerged healing of ligature induced peri-implantitis lesions: an experimental study in dogs

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Abstract

Objective: The aim of the present study was to evaluate non-submerged and submerged healing of ligature induced peri-implantitis in dogs.

Material and Methods: Peri-implantitis was induced by ligature placement in five beagle dogs ($n = 30$ implants). The defects were randomly and equally allocated in a split-mouth design to either closed treatment+non-submerged healing (CNS), or open treatment+submerged healing (OS) using an Er:YAG laser (ERL), an ultrasonic device (VUS), or plastic curettes+local application of metronidazole gel (PCM), respectively. The animals were sacrificed after 3 months. Clinical, radiological and histological (e.g. new bone-to-implant contact (BIC)) parameters were assessed.

Results: All treatment procedures resulted in statistically significant improvements of all clinical parameters at both CNS and OS implants. Radiological improvements were merely observed at OS implants. Histomorphometrical analysis revealed that all CNS implants exhibited comparable low amounts of new BIC (1.0–1.2%), while mean BIC was statistically significant higher in the respective OS groups [ERL (44.8%), PCM (14.8%), VUS (8.7%)].

Conclusion: Within the limits of the present study, it was concluded that (i) OS improved the outcome of treatment in comparison with CNS and (ii) ERL seemed to be more suitable to promote re-osseointegration than PCM and VUS.

Key words: animal study; dental implant; Er:YAG laser; ligature induced; metronidazole; peri-implantitis; ultrasonic device

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Peri-implant disease is a collective term for 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 supporting alveolar bone (Albrektsson & Isidor 1994). Nowadays, there is considerable evidence for the microbial etiology of peri-implant diseases (Mombelli et al. 1987, 1988, Becker et al. 1990, Alcoforado et al. 1991). Indeed, a progressive inflammation and a rapid breakdown of peri-implant soft and hard tissues has been observed in an experimental peri-implantitis model in both dogs and monkeys. In this model, peri-implantitis lesions were induced by terminating the plaque control regimen, and placement of cotton ligatures submarginally around implants (Lindhe et al. 1992, Lang et al. 1993, Schou et al. 1993, Marinello et al. 1995, Baron et al. 2000, Zitzmann et al. 2004). As peri-implantitis was also classified as a

disease process associated with micro-organisms known from chronic periodontitis (Mombelli et al. 1987, Rams et al. 1991, Leonhardt et al. 1999), it was assumed that the removal of bacterial plaque biofilms from the implant surface may be a pre-requisite in order to stop disease progression. In recent years, various therapies such as access flap surgery, debridement and chemical conditioning of the implant surface, topical or systemic antibiotic and/or antimicrobial therapy, and bone regenerative procedures have been advocated for both the resolution of the peri-implant infection and the restoration of the implant supporting tissues (Ericsson et al. 1996, Persson et al. 1996, Hürzeler et al. 1997, Leonhardt et al. 2003, Roos-Jansaker et al. 2003, Schou et al. 2003a–c, 2004). Even though clinically healthy peri-implant tissues were obtained and maintained in most of these studies, the amount of documented bone regeneration and re-osseointegration varied considerably and has even been questioned (Schou et al. 2004). Several factors have been discussed to explain the lack of re-osseointegration at contaminated implants. First of all, the removal of bacterial plaque biofilms from structured implant surfaces using conventional mechanical treatment procedures including plastic curettes and sonic/ultrasonic scalers has been shown to be incomplete (Kreisler et al. 2005, Schwarz et al. 2005b, 2006). Although air-powder-flow was also successfully used for implant surface decontamination in vitro (Augthun et al. 1998, Kreisler et al. 2005), there are limitations in the application because it can lead to microscopically visible alterations of the implant surface and be associated with an increased risk of emphysema (Parham et al. 1989, Van de Velde et al. 1991, Kreisler et al. 2005). Recently, a newly developed ultrasonic device (Vector™, VUS, Dürr Dental, Bietigheim-Bissingen, Germany) has been introduced in order to facilitate the removal of bacterial deposits from implant surfaces (Hahn 2000, Karring et al. 2005, Schwarz et al. 2005b). The energy from the vertical vibration of the instrument is transmitted to the implant surface and the peri-implant tissues by a suspension of hydroxyapatite (HA) particles and water. Subsequently, the removal of bacterial deposits is supposed to be achieved because of hydrodynamic forces rather than by the chipping action of the working tip (Hahn 2000, Braun et al. 2005). In addition to mechanical treatment approaches, the use of different laser systems has also been investigated for treatment of peri-implant infections (Deppe et al. 2001, Persson et al. 2004). Close attention has been paid to the clinical applicability of the Er:YAG laser (ERL) with a wavelength of 2.940 nm in the near infrared spectrum. Preliminary experimental and clinical results have also shown, that this type of laser seemed to be capable of effectively removing bacterial deposits from both smooth and rough titanium implants without injuring their surfaces (Matsuyama et al. 2003, Schwarz et al. 2003b, 2005b, Kreisler et al. 2005). Although preliminary clinical data suggest that closed treatment of peri-implantitis with both ERL and VUS may lead to significant clinical improvements as evidenced by a reduction of bleeding on probing (BOP) and probing pocket depths (PD) (Karring et al. 2005, Schwarz et al. 2005a), there are currently no histologic data available evaluating the influence of both treatment procedures on peri-implant wound healing. In this context, it must also be queried whether open treatment and a submerged healing procedure may improve the outcome of therapy in comparison with closed instrumentation.

Therefore, the aim of the present study was to evaluate clinical, radiological and histological changes at ligatur-induced peri-implantitis defects in dogs following closed treatment+non-submerged healing, or open treatment+submerged healing using either ERL, VUS, or plastic curettes and local application of metronidazole gel (PCM).

Material and Methods

Animals

Five 6-year-old female beagle dogs (mean weight 16.3 kg) were used in the study. All animals exhibited a fully erupted permanent dentition. During the experiment, the dogs were fed once per day with soft-food diet and water. Animal selection, management and surgery protocol were approved by the Animal Care and Use Committee of the Heinrich Heine University and the Bezirksregierung Düsseldorf.

Ligature induced peri-implantitis

The experimental segment of the study started after an adaptation period of 4 weeks. The study was performed in three surgical phases. In the first phase, extraction of the mandibular second, third, fourth pre-molar and 1st molar (P2-M1) was performed bilaterally. After 4 months of healing, surgical implantation of screw-typed implants was performed according to a one-stage healing procedure during the second phase. Following implant installation, a plaque control programme was initiated. Tooth and implant cleaning were maintained by the use of a toothbrush once a day for 3 months. Radiographs were obtained before and immediately after tooth extraction as well as immediately after implant installation. After 3 months of healing, cotton ligatures were placed in a submarginal position around each implant according to a method previously described (Lindhe et al. 1992) and the plaque-control regimen was terminated. In brief, ligatures were forced into a position directly apical of the gingival margin. Subsequently, a ''pocket'' was created which enabled the establishment of a subgingival microflora (Fig. 1). The ligatures were exchanged once every 3 weeks and removed when approximately 40% of the initial bone support was lost (approximately 3 months) based on evaluation of standardized radiographs.

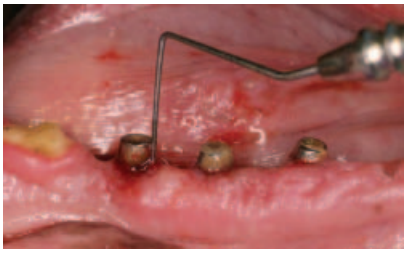

Fig. 1. Acute signs of peri-implant infections 4 weeks after ligature removal.

Four weeks after ligature removal, the defects were randomly and equally allocated in a split-mouth design to either closed treatment+non-submerged healing (CNS), or open treatment+submerged healing (OS) using ERL, VUS or PCM.

Randomization was performed according to a computer generated protocol (RandList®, DatInf GmbH, Tübingen, Germany). For allowing randomization, supra- and intra-bony components were estimated before treatment on radiographs. The randomization process led to comparable mean values of all investigated clinical parameters at baseline in all groups. Accordingly, in

each animal, the implants of both CNS and OC groups received each treatment procedure.

Surgical procedures

After sedation with acepromazine (0.17 mg/kg body weight), the dogs were anaesthetized with 21.5 mg/kg thiopental-sodium. For all surgical procedures, inhalation anaesthesia was performed by use of oxygen and nitrous oxide and isoflurane. To maintain hydration, all animals received a constant rate infusion of lactated Ringer's solution while anaesthetized.

In the first surgery, P2-M1 were carefully removed after reflection of full-thickness mucoperiosteal flaps and tooth separation. After wound closure by means of matress sutures, the sites were allowed to heal for 4 months. Prophylactic administration of clindamycine (11.0 mg/kg body weight, Cleorobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and postoperatively for 10 days.

In the second surgery, bilateral vestibular incisions were made and full-thickness mucoperiosteal flaps were elevated to expose the respective sites for implant placement in the mandible. Three surgical implant sites were prepared bilaterally, at a distance of 8 mm apart, using a low-trauma surgical technique under copious irrigation with sterile 0.9% physiological saline. Three sand-blasted, large grit and acid-etched (SLA) titanium implants were placed in each side of the mandible (narrow neck, ∅3.3 mm, length 10 mm, Institut Straumann® AG, Basel, Switzerland) ($n = 6$ implants per dog) according to a one-stage procedure and covered with healing abutments (height: 2 mm, Institut Straumann® AG). The implants were inserted in a way so that the borderline between the bony and transmucosal part (BTB) of the implant coincided with the bone crest. Following irrigation, mucoperiosteal flaps were repositioned and primary wound closure was achieved with consecutive resorbable 5.0 polyglygolic acid sutures (Resorba, Nürnberg, Germany).

In the third surgery, bilateral intrasulcular and vertical releasing incisions were made and full-thickness mucoperiosteal flaps were reflected to expose the respective peri-implant bone defects in the OS groups. Following replacement of the healing abutments by cover screws (Institut Straumann® AG), the granulation tissue was removed from the defects and the respective implant surfaces were instrumented using either ERL, VUS or PCM. Finally, mucoperiosteal flaps were extended coronally to facilitate a submerged healing procedure. Primary wound closure was achieved with consecutive polyglycolic acid 5.0 Polyester® sutures (Resorba). In the CNS groups, healing abutments were autoclaved, followed by a closed instrumentation of the respective implant surfaces. Postoperative care consisted of oral hygiene procedures including tooth and implant brushing and was performed four times a week for the rest of the study period of 3 months.

The animals were sacrificed (overdose of sodium pentobarbital 3%) after a healing period of 3 months and the oral tissues were fixed by perfusion with 10% buffered formalin administered through the carotid arteries. The jaws were dissected and blocks containing the experimental specimens were obtained. All specimens were fixed in 10% neutral buffered formalin solution for 4–7 days.

Treatment procedures

At the start of the third surgery, each dog received a supragingival cleaning of implants and remaining teeth in the upper and lower jaws using rubber cups and a polishing paste (Zircate® Prophy Paste, Dentsply, Konstanz, Germany), followed by a subgingival instrumentation of periodontally diseased root surfaces.

An ERL device (KEY3®, KaVo, Biberach, Germany) emitting a pulsed infrared radiation at a wavelength of 2.940 nm was selected for laser treatment. The laser beam was guided onto the implant surfaces under water irrigation with a specially designed periodontal handpiece and a cone-shaped glass fibre tip emitting a radial and axial laser beam (2061, KaVo). Laser parameters were set at 100 mJ/pulse (12.7 J/cm^2), 10 Hz, and pulse energy at the tip was approximately 85 mJ/pulse (Schwarz et al. 2003a, b, 2005a, b). The fibre tip was guided in a semicircular motion from coronal to apical parallel to the implant surface in contact mode.

The VUS system was used with a straight polyether ethercetone fibre (PEEK) and a polishing fluid (HA particles < 10 µm) (Vector®) according to the instructions given by the manufacturer (70% power setting).

PCM was performed using plastic curettes (Institut Straumann® AG), followed by subgingival (i.e. CNS groups) or peri-implant (i.e. OS groups) application of metronidazole gel 25% (Elyzol®, Colgate-Palmolive, Hamburg, Germany).

All surgical procedures were performed by one investigator well trained in peri-implantitis treatment using all procedures employed in the present study.

In all groups, instrumentation was carried out until the operator felt that the granulation tissue was completely removed from the intra-bony peri-implantitis defects (i.e. OS group) and the implant surfaces were adequately debrided (i.e. CNS and OS groups). This was evaluated by tactile sensation with the respective fibres in the ERL and VUS groups and with a periodontal probe (PCP12, Hu-Friedy Co., Chicago, IL, USA) in the PCM group (Fig. 2).

Clinical measurements

The following clinical parameters were measured immediately before ligature placement, as well as before and 3 months after treatment using the same PCP12 periodontal probe: (1) BOP, evaluated as present if bleeding was evident within 30 s after probing, or absent, if no bleeding was noticed within 30 s after probing, (2) PD measured from the mucosal margin to the bottom of the probeable pocket, (3) gingival recession (GR) measured from the implant shoulder (IC) to the mucosal margin and (4) clinical attachment level (CAL) measured from IC to the bottom of the probeable pocket. The measurements were made at five aspects per implant: mesiobuccal (mb), midbuccal (b), distobuccal (db), mesiolingual (ml) and distolingual (dl).

Radiographic observations

Standardized radiographs were obtained from each implant site at ligature placement (baseline), at each ligature exchange, as well as immediately before and 3 months after treatment using individually adjusted film holder devices. Digital images of the radiographs (original magnification × 200) were evaluated using a software program (analySIS FIVE docu®, Soft Imaging System, Münster, Germany) by

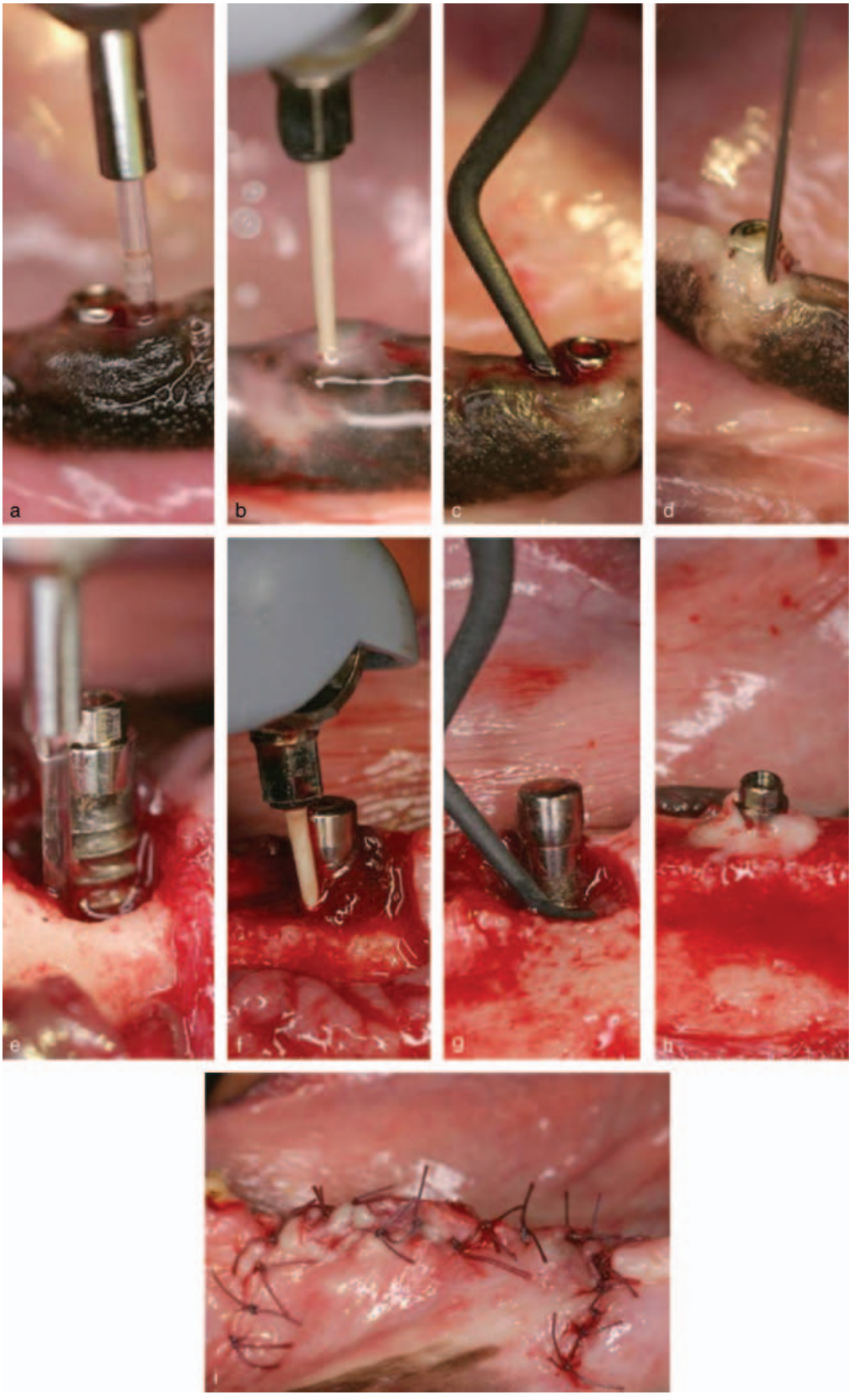

Fig. 2. Peri-implantitis lesions were randomly and equally allocated in a split-mouth design to either closed treatment+non-submerged healing (a–d), or open treatment+submerged healing (e–i) using Er:YAG laser (a and e), an ultrasonic device (b and f) or plastic curettes+local application of metronidazole gel (c, d, g and h), respectively.

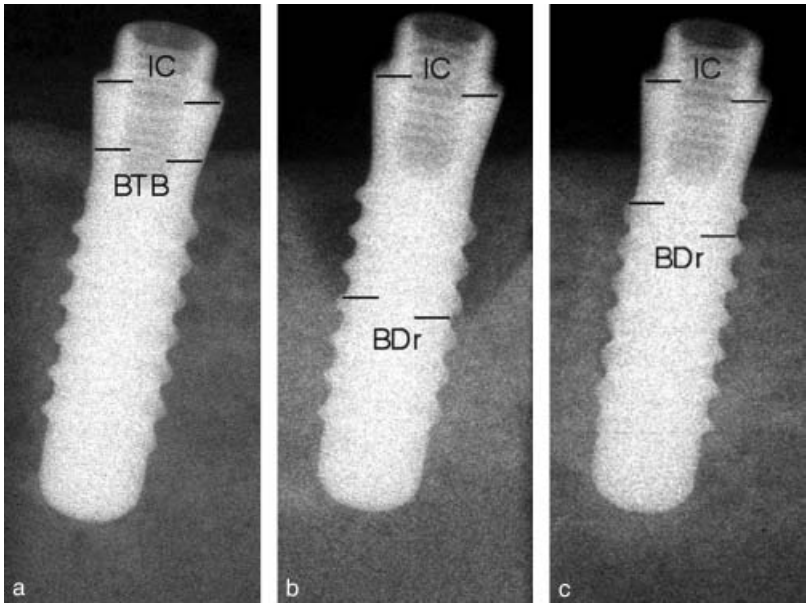

Fig. 3. Standardized radiographs were used to assess the distance between the implant shoulder (IC) and the bottom of the bone defect (BDr) next to the implant at the mesial and distal surfaces. (a) Defect characteristic immediately after implant installation. (b) Ligature-induced peri-implant bone loss – situation immediately before (b), and 3 months after treatment in the PCM–OS group (c) (corresponding histological view is illustrated in Fig. 5).

assessing the distance between IC and the bottom of the bone defect at the mesial and distal surfaces at implant placement (BDr0), as well as immediately before (BDr1), and 3 months following treatment (BDr3) (Fig. 3).

Configuration assessment of peri-implant bone defects

During open-flap surgery in the OS groups, the following measurements were carried out at the mesial (m), distal (d) and b aspects of each implant using the same PCP12 periodontal probe: (a) maximum linear distance from IC to the bottom of the bone defect (BDc), and (b) supraalveolar component of the defect, measured as maximum linear distance from BTB to the alveolar bone crest (BC).

Histological preparation

The specimens were dehydrated using ascending grades of alcohol and xylene, infiltrated, and embedded in methylmethacrylate (MMA, Technovit 7200, Heraeus Kulzer, Wehrheim, Germay) for non-decalcified sectioning. After 20 h the specimens were completely polymerized. Each implant site was cut in the mesio-distal direction along with the long axis of the implant using a diamond wire saw (Exakt®, Apparatebau, Norderstedt, Germany). Additionally, the buccal part of each implant site was cut in bucco-lingual direction. Serial sections were prepared from both the mesio-distal and bucco-lingual parts, resulting in three sections of approximately 500 μm in thickness each (Donath 1985). Subsequently, all specimens were glued with acrylic cement (Technovit 7210 VLC, Heraeus Kulzer) to opaque plexiglas and ground to a final thickness of approximately 30 μm. All sections were stained with toluidine blue (TB) to evaluate new bone formation. With this technique, old bone stains light blue, whereas newly formed bone stains dark blue because of its higher protein content (Schenk et al. 1984).

Histomorphometrical analysis

For image acquisition a colour CCD camera (Color View III, Olympus, Hamburg, Germany) was mounted on a binocular light microscope (Olympus BX50, Olympus). Digital images (original magnification $\times$ 200) were evaluated using a software program (analySIS FIVE docu®, Soft Imaging System).

The following landmarks were identified in the stained sections: IC, the marginal portion of the peri-implant mucosa (PM), the apical extension of the long-junctional epithelium (aJE), the apical extension of the inflammatory cell infiltrate (aICT), the most coronal level of bone in contact with the implant (CBI), the bottom of the bone defect (BDh), and the level of the lateral bone wall of the defects (BC). The landmarks are illustrated in Figs 4–6.

Bone regeneration was defined as (IC–BDh)–(IC–CBI), bone defects width as linear distance between BC and the implant surface, and bone defects depth as (IC–BDh)–(IC–BC). For each implant site, the reduction of the osseous defect was expressed as percentage of bone regeneration/bone defects depth. Furthermore, the amount of new BIC was measured as percentage of the distance BDh to BTB. All measurements were made at the m, d, and b implant sites of each specimen.

Intra-examiner reproducibility

All clinical, radiological and histomorphometrical measurements were performed by one experienced investigator masked to the specific experimental conditions. Clinical calibration (PD, GR and CAL) was performed at five patients attending the Department of Oral Surgery, Heinrich Heine University, Düsseldorf, Germany for peri-implantitis treatment procedures. Each patient exhibited two implants with PD $>$ 6 mm on at least one aspect of the implant. Radiological/histomorphometrical calibration was performed by means of five radiographs/histological sections. The examiner evaluated the patients/specimens on two separate occasions, 48 h apart. Calibration was accepted if measurements at baseline and at 48 h were similar at $>$ 90% level.

Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS® 14.0, SPSS Inc.). Mean values of all parameters were calculated. Normal distribution was looked for by the Kolmogorov–Smirnow test. Analysis of variance (ANOVA) and posthoc testing using Bonferroni's correction for multiple comparisons was used for comparisons between groups (i.e. comparisons of either CNS or OS implants between different treatment groups). The paired *t*-test was used to compare values within groups (i.e. comparisons of CNS and OS implants as well as comparison of mean clinical and radiological values obtained at baseline and after 3 months of healing within

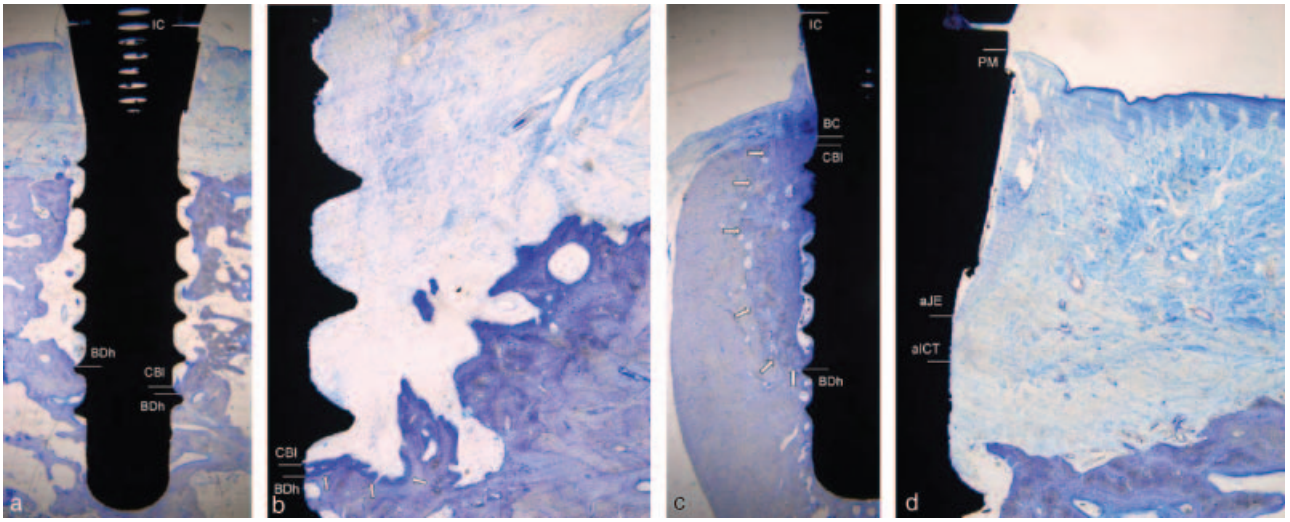

Fig. 4. Representative histologic views of peri-implant wound healing in the VUS groups. A direct contact between the newly formed bone and the implant surface was generally limited to the most apical part of the defects in both closed treatment+non-submerged healing (CNS) and open treatment+submerged healing (OS) groups (a, b). One specimen also exhibited new bone-to-implant contact (BIC) in the coronal third of the formerly bone defect area at the b aspect of the implant surface (c). The newly formed connective tissue zone exhibited considerable amounts of inflammatory cells (d). (a) m/d aspect, OS group; original magnification × 12.5 – for corresponding clinical view see Fig. 2f). (b) d aspect of Fig. 3a, CNS group; original magnification × 40). (c) b aspect of Fig. 3a, OS group; original magnification × 40). (d) d aspect, CNS group; original magnification × 40). Arrows indicate the former defect dorders

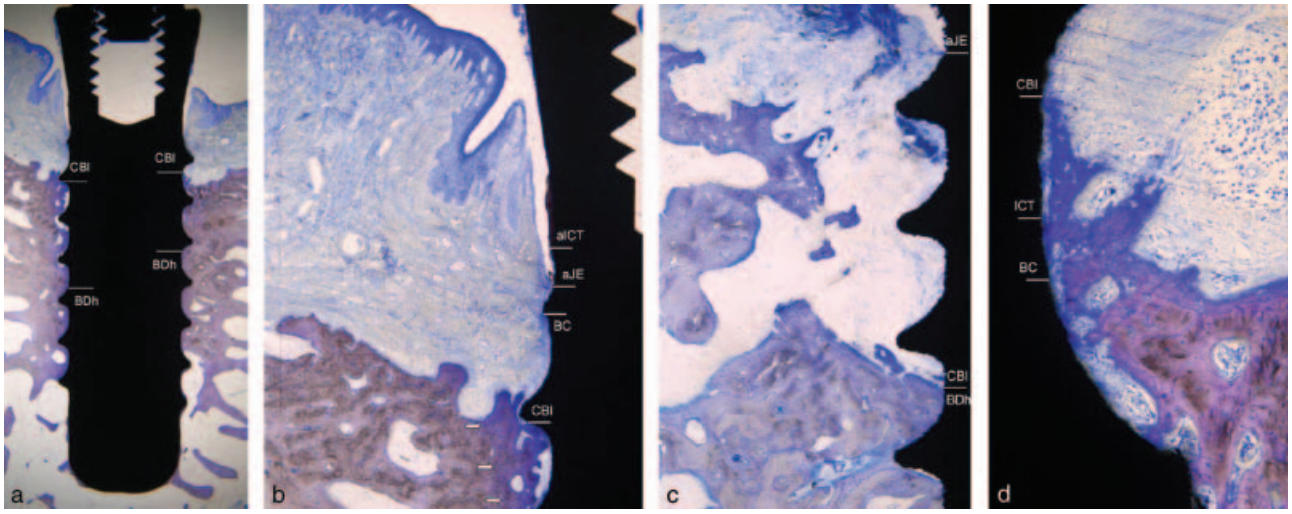

Fig. 5. Representative histologic views of peri-implant wound healing in the plastic curettes+local application of metronidazole gel (PCM) groups. The PCM–open treatment+submerged healing (OS) group revealed significantly higher amounts of re-osseointegration than the respective PCM–closed treatment+non-submerged healing (CNS) group (a–c). However, the newly formed bone was frequently separated from the implant surface by a connective tissue formation (d). (a) m/d aspect, OS group; original magnification × 12.5 – for corresponding clinical view see Fig. 2g. (b) m aspect of Fig. 4a, OS group; original magnification × 40. (c) m aspect, CNS group; original magnification × 40. (d) d aspect of Fig. 4a, OS group; original magnification × 100. Arrows indicate the former defect dorders

each treatment group). Significance was set at $p = 0.05$ after adjusting for multiple comparisons.

Results

Clinical measurements

The postoperative healing was uneventful in all cases. No complications such as abscesses or acute inflammatory reactions were observed throughout the study period. Furthermore, there were no signs of any adverse effects that could be associated with the respective treatment procedures. However, between 8 and 10 weeks following primary wound closure in the OS groups, all implants had prematurely penetrated the mucosa.

Mean values of BOP, PD, GR and CAL at baseline and 3 months following treatment in all groups are summarized in Table 1. At the baseline examination, there were no statistically significant differences in any of the investigated parameters between the groups (ANOVA, $p > 0.05$, respectively). However, with respect to GR, it was noted that all implants exhibited an inflamed hyper-

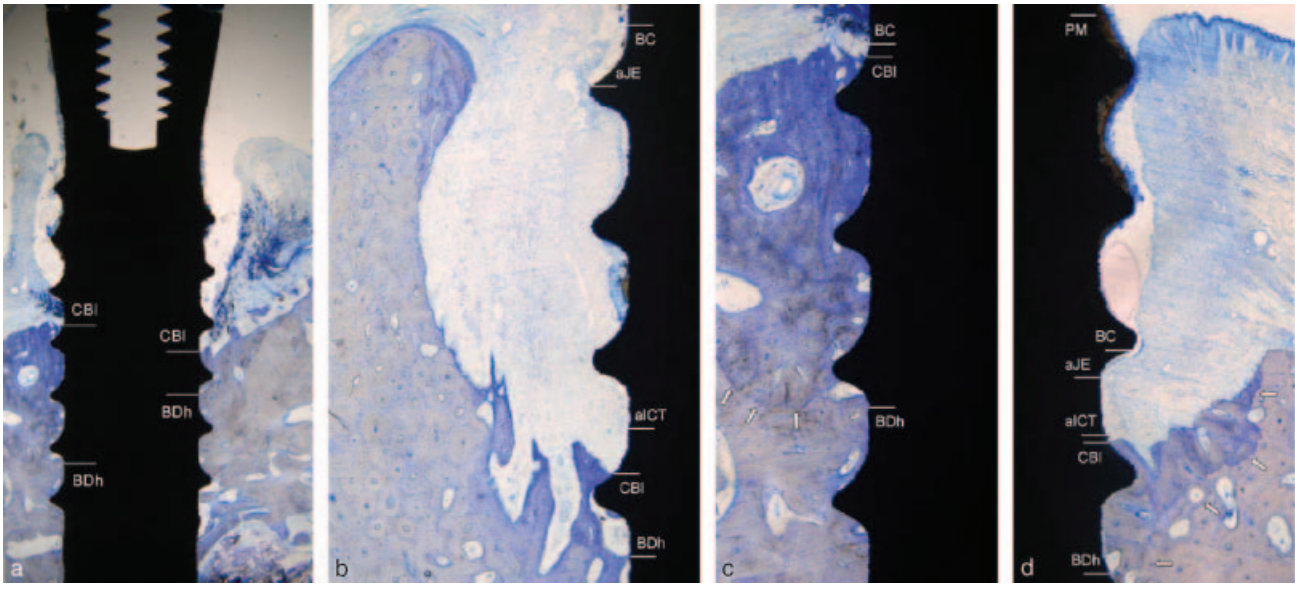

Fig. 6. Representative histologic views of peri-implant wound healing in the Er:YAG laser (ERL) groups. The ERL-open treatment+submerged healing (OS) group also exhibited significantly higher amounts of re-osseointegration than the respective ERL–closed treatment+non-submerged healing (CNS) group (a, b). However, in contrast to the plastic curettes+local application of metronidazole gel (PCM)–OS group, the newly formed bone seemed to be firmly attached to the respective implant surfaces in the ERL–OS group (c). The presence of inflammatory cells in the connective tissue zone seemed to be correlated with the presence of residual or newly formed subgingival calculus (d). (a) m/d aspect, OS group; original magnification × 12.5 – for corresponding clinical view see Fig. 2e. (b) b aspect, CNS group; original magnification × 40. (c) m aspect of Fig. 4a, OS group; original magnification × 40. (d) d aspect of Fig. 4a, OS group; original magnification × 40. Arrows indicate the former defect dorders.

Table 1. Clinical Parameters

	CNS			OS		
	ERL	VUS	PCM	ERL	VUS	PCM
BOP (0)	92	90	93	88	84	94
BOP (3)	54	77*	50	49	53	49
PD (0)	5.0 ± 0.8	5.1 ± 0.9	5.1 ± 0.8	5.1 ± 1.5	5.1 ± 1.1	5.0 ± 1.2
PD (3)	4.3 ± 0.5	4.9 ± 1.0	4.3 ± 0.3	4.0 ± 1.1	4.1 ± 0.7	4.4 ± 1.3
GR (0)	+0.9 ± 0.5	+0.9 ± 0.4	+1.0 ± 0.6	+0.9 ± 0.4	+1.1 ± 0.6	+1.0 ± 0.2
GR (3)	0.5 ± 0.3	0.3 ± 0.2	0.4 ± 0.3	+0.9 ± 1.1	+0.8 ± 1.0	+1.3 ± 0.8
CAL (0)	4.1 ± 0.9	4.3 ± 1.0	4.1 ± 1.0	4.1 ± 1.6	4.0 ± 1.1	4.1 ± 1.3
CAL (3)	4.8 ± 1.0	5.2 ± 1.0**	4.7 ± 0.9	3.1 ± 0.6	3.3 ± 0.9	3.1 ± 1.8

*Statistically significant lower compared with ERL–CNS and PCM–CNS (ANOVA, $p<0.05$).
**Statistically significant lower compared with VUS–OS (paired *t*-test, $p<0.01$).
Bleeding on probing (BOP in %), probing depth (PD), gingiva recession (GR), and clinical attachment level (CAL) (in mm). mean scores (measured at 5 sites per implant ± SD) at baseline (0), and 3 months in different groups ($n = 30$ implants).
CNS, treatment+non-submerged healing; OS, treatment+submerged healing; ERL, Er:YAG laser; VUS, ultrasonic device; PCM, metronidazole gel.
+ = Hyperplasia.

plastic peri-implant gingiva at baseline. After 3 months of healing in both CNS and OS groups, all treatment procedures resulted in statistically significant improvements of all clinical parameters. In particular, mean BOP scores improved statistically significant at both CNS and OS implants in all treatment groups (paired *t*-test, $p<0.001$, respectively). However, mean reduction of BOP scores in the VUS–CNS group was statistically significant lower compared with both ELR–CNS and PCM–CNS groups (ANOVA, $p<0.05$, respectively). The BOP reductions within the respective treatment groups were statistically non-significant different at CNS and OS implants (paired *t*-test, $p>0.05$).

After 3 months of healing, all treatment procedures resulted in statistically significant reductions of PD, increases of GR, and gains of CAL (paired *t*-test, $p<0.001$, respectively) at both CNS and OS implants (Table 1). However, statistical analysis revealed that in the VUS group, mean CAL gains were statistically significant higher at OS when compared with CNS implants (paired *t*-test, $p<0.01$). In contrast, ERL and PCM groups revealed comparable mean CAL gains at both CNS and OS implants (paired *t*-test, $p>0.05$, respectively).

Configuration and sizes of ligature-induced peri-implantitis defects in the OS groups are summarized in Table 2. In particular, statistical analysis revealed no significant differences in terms of mean IC–BDc and BTB–BC values between ERL, VUS and PCM groups (ANOVA, $p>0.05$, respectively).

Radiological measurements

Mean values of IC–BDr at ligature placement, as well as immediately before and 3 months following treatment in different groups are summarized in Table 3. In particular, at ligature placement statistical analysis revealed no significant differences in terms of mean IC–BDr(0) values within or between groups (paired *t*-test, ANOVA, $p>0.05$, respectively). Ligature placement resulted in statistically significant

Table 2. Clinical Parameters

	ERL	VUS	PCM
IC–BDc	5.3 ± 1.4	5.1 ± 0.8	5.3 ± 1.1
BTB–BC	1.1 ± 1.0	0.7 ± 0.5	0.8 ± 0.4

Configuration and size of ligature-induced peri-implantitis bone defects in the OS- groups. Mean scores measured at mesial, distal and buccal sites per implant (± SD in mm) ($n = 30$ implants). IC, implant shoulder; BDc, bottom of the bone defect; BTB, bony and transmucosal part; BC, bone crest; ERL, Er:YAG laser; VUS, ultrasonic device; PCM, metronidazole gel.

Table 3. Radiological Parameters

	CNS			OS		
	ERL	VUS	PCM	ERL	VUS	PCM
IC–BDr (0)	2.9 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	2.8 ± 0.1
IC–BDr (1)	5.0 ± 1.2	4.9 ± 0.7	5.0 ± 0.6	5.6 ± 1.0	5.5 ± 1.0	5.9 ± 1.3
IC–BDr (3)	4.8 ± 0.5	4.6 ± 0.5	4.8 ± 0.5	3.9 ± 0.8	4.9 ± 0.8	5.1 ± 1.8

Distance between the implant shoulder (IC) and the bottom of the bone defect (BDr) next to the implant at the mesial and distal surfaces. Mean scores (± SD in mm) at ligature placement (0), immediately before (1) and 3 months following treatment in different groups ($n = 30$ implants). CNS, treatment+non-submerged healing; OS, treatment+submerged healing; ERL, Er:YAG laser; VUS, ultrasonic device; PCM, metronidazole gel; IC, implant shoulder; BDr, bone defect at the mesial and distal surfaces.

Table 4. Histomorphometrical Parameters

	CNS			OS		
	ERL	VUS	PCM	ERL	VUS	PCM
PM–aJE	1.6 ± 0.4	1.7 ± 0.6	1.5 ± 0.5	2.7 ± 2.0	2.2 ± 1.2	2.6 ± 0.6
aJE–CBI	2.9 ± 0.5	3.4 ± 1.3	2.8 ± 0.6	1.5 ± 0.7	2.7 ± 0.8	2.2 ± 1.7
PM–aICT	0.9 ± 0.6	1.6 ± 0.8	1.3 ± 0.9	1.4 ± 1.2	1.0 ± 0.8	1.3 ± 0.8
IC–BDh	4.9 ± 1.0	5.5 ± 1.3	4.5 ± 0.4	5.5 ± 1.7	5.3 ± 1.2	5.7 ± 1.6
IC–CBI	4.5 ± 0.3	5.0 ± 1.3	4.4 ± 0.4	4.4 ± 1.4	4.8 ± 1.1	4.8 ± 1.5
IC–BC	3.2 ± 0.5	3.2 ± 0.4	3.0 ± 0.1	3.9 ± 0.9	3.5 ± 1.0	3.6 ± 0.5
Bone defects depth (IC–BDh)–(IC–BC)	1.7 ± 0.7	2.3 ± 1.3	1.6 ± 0.4	1.6 ± 1.3	1.8 ± 1.3	2.1 ± 1.7
Bone defects width BC implant	1.8 ± 0.6	1.9 ± 0.5	1.9 ± 0.5	2.0 ± 0.6	2.0 ± 0.5	1.9 ± 0.6
Bone regeneration (IC–BDh)–(IC–CBI)	0.4 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	1.3 ± 1.0*	0.5 ± 0.4	0.9 ± 0.7*
Reduction of the osseous defect (%)	23.1	15.4	15.4	77.8	26.4	44.6
BIC (%)	1.1	1.2	1.0	44.8*	8.7**	14.8*

*Statistically significant higher compared with ERL–CNS and PCM–CNS (paired *t*-test, $p < 0.001$, respectively), and VUS–OS (ANOVA, $p < 0.001$, respectively).

**Statistically significant higher compared with VUS-CNS (paired *t*-test, $p < 0.05$, respectively).

Mean scores (measured at mesial, distal and buccal sites per implant ± SD in mm) after 3 months of healing ($n = 30$ implants).

CNS, treatment+non-submerged healing; OS, treatment+submerged healing; ERL, Er:YAG laser; VUS, ultrasonic device; PCM, metronidazole gel; PM, peri-implant mucosa; aJE, long-junctional epithelium; IC, implant shoulder; CBI, bone in contact with the implant; aICT, apical extension of the inflammatory cell infiltrate; BDh, bottom of the bone defect; BC, bone crest.

increased IC–BDr values at both mesial and distal aspects of each implant (paired *t*-test, $p < 0.001$, respectively). Although mean IC–BDr(1) values tended to be highest in the PCM–OS group, this difference did not reach statistical significance (ANOVA, $p > 0.05$, respectively). Subsequent to instrumentation, all treatment procedures resulted in statistically significant decreases of mean IC–BDr(3) values at OS implants (paired *t*-test, $p < 0.01$, respectively). Even though these changes tended to be highest in ERL and PCM groups, the differences did not reach statistical significance (ANOVA, $p > 0.05$, respectively). In contrast, mean decreases of IC–BDr(3) values were statistically non-significant at CNS implants in all groups (paired *t*-test, $p > 0.05$, respectively) (Table 3).

Histological measurements

Mean values of all histomorphometrical parameters are summarized in Table 4. The conditions of the peri-implant soft and hard tissues adjacent to SLA implants in different treatment groups are illustrated in Figs 4–6.

In particular, morphometrical analysis at the soft-tissue interface revealed that mean values of PM–aJE and aJE–CBI were comparable at respective CNS and OS implants in all treatment groups (ANOVA, $p > 0.05$, respectively). However, it was also observed that mean values of PM–aJE were statistically significant higher at OS implants in comparison with CNS implants (paired *t*-test, $p < 0.01$, respectively) (Figs 4–6). In both CNS and OS groups, CBI was clearly separated from aJE by a dense, well-vascularized connective tissue (aJE–CBI). This connective tissue appeared to be in close contact with the respective implant surfaces (Figs 4b, 5b and 6b). However, this zone was also characterized by an infiltration of inflammatory cells (i.e. macrophages, multinucleated giant cells), as demonstrated by comparable mean values of PM–aICT between and within groups (ANOVA, paired *t*-test; $p > 0.05$, respectively). This seemed to be particularly true for implant sites and surfaces exhibiting residual or newly formed subgingival calculus, which was commonly observed in all groups (Figs 4d, 5b and 6d). Statistical analysis of mean IC–BDh values, bone defect widths (BC–Impl), and bone defect depths [(IC–BDh)–(IC–BC)] revealed no significant differences between or within groups (ANOVA, paired *t*-test, $p > 0.05$, respectively).

Histological observation showed characteristic healing patterns of the peri-implant hard tissues in the different groups. In particular, all CNS groups and the VUS–OS group were mainly characterized by the formation of a long-junctional epithelium and a dense connective tissue zone along the instrumented implant surfaces. Minute amounts of new bone formation were observed only occasionally, and were limited to the most apical part of the respective defects (Figs 4a, b, 5c and

6b). In contrast, OS implants of the ERL and PCM groups exhibited statistically significant higher amounts of new bone formation along the instrumented implant surfaces (paired *t*-test, $p < 0.001$, respectively) (Figs 5a–c and 6a–c). However, new bone formation was limited to the intra-bony component of the former defect borders. Statistical analysis of bone regeneration [(IC–BDh)–(IC–CBI)] revealed significantly higher values for ERL–OS and PCM-OS groups in comparison to the VUS–OS group (ANOVA, $p < 0.001$, respectively). However, the difference between ERL–OS and PCM–OS was statistically non-significant (ANOVA, $p > 0.05$, respectively). Histomorphometrical analysis of new BIC revealed obvious differences between groups. In particular, CNS implants of ERL, VUS and PCM groups generally exhibited comparable low amounts of new BIC, ranging from 1.0% to 1.2% (ANOVA, $p > 0.05$, respectively). In contrast, mean BIC values were statistically significant higher in the respective OS groups [i.e. ERL (44.8%), PCM (14.8%) and VUS (8.7%)] (paired *t*-test, $p < 0.001$, $p < 0.05$, $p < 0.001$, respectively). Histological observation of the former bone defect area in the ERL–OS group revealed, that the newly formed bone exhibited a mature, parallel-fibered structure, which seemed to be firmly attached to the respective implant surfaces (Figs 6c and d). The maturity of this woven bone was also identifiable by the development of primary osteons. In contrast, the PCM–OS group revealed greater amounts of a connective tissue formation separating the newly formed bone from the implant surface (Figs 5b and d). Histomorphometrical analysis of new BIC at OS implants revealed significantly lowest values in the VUS group (ANOVA, $p < 0.001$, respectively), where a direct contact between the newly formed bone and the implant surface was generally limited to the most apical part of the defects (Fig. 4a). However, one specimen in the VUS-OS group also exhibited new BIC in the coronal third of the formerly bone defect area at the b aspect of the implant surface (Fig. 4c).

Correlation between clinical, radiological and histological measurements

In general, clinical assessment of PD tended to pass beyond the histological level of aJE at b and m/d sites of both CNS and OS implants. In particular, mean values of PD(3) were statistically significant higher than respective PM-aJE values (paired *t*-test, $p < 0.01$, respectively). This seemed to be particularly true for sites exhibiting higher mean values of PM–aICT (i.e. m/d sites in all groups).

At both CNS and OS implants of all treatment groups, radiological examination identified BD and CBI close to the histological level. In particular, at m/d-implant sites, mean values of IC–BDr(1) and IC–BDr(3) seemed to be within the range of respective IC–BDh and IC–CBI values (paired *t*-test, $p > 0.05$, respectively).

Discussion

The results of the present study have indicated that treatment of ligature-induced peri-implantitis lesions using ERL, VUS and PCM resulted in clinically and histologically important improvements of all investigated parameters. The observation that CNS treatment of peri-implantitis lesions using ERL may lead to statistically significant BOP reductions and CAL gains is in agreement with previous findings (Schwarz et al. 2005a). In particular, it was reported that mean value of BOP decreased in the ERL group from 83% at baseline to 31% after 6 months, and mean CAL changed from 5.8 ± 1 mm at baseline to 5.1 ± 1.1 mm after 6 months. In contrast, mechanical debridement using plastic curettes and antiseptic therapy with chlorhexidine digluconate (PC) resulted in a decrease of mean BOP from 80% at baseline to 58% after 6 months, and a change of mean CAL from 6.2 ± 1.5 mm at baseline to 5.6 ± 1.6 mm after 6 months. The difference between both groups with respect to BOP reduction was statistically significant (Schwarz et al. 2005a). The BOP reductions and CAL gains following closed treatment with VUS of the present study are also in agreement with previous findings (Karring et al. 2005). Even though there was a greater reduction in the number of sites with BOP following treatment with VUS than following instrumentation with PC, there was no significant difference between the two methods. Furthermore, in both groups there were no differences between baseline and 6 months regarding PD reductions and bone level changes (Karring et al. 2005). In this context, however, it must be pointed out that Karring et al. (2005) used the VUS system in combination with a carbon fibre tip. The reason for choosing the PEEK fibre in the present study was based on the observation that mechanical instrumentation of titanium surfaces using the carbon fibre resulted in attrition and deposition of this specific material, which in turn disturbed attachment of osteoblast-like cells (Schwarz et al. 2003a). To the best of our knowledge, these are the first data evaluating PCM for both CNS and OS treatment of peri-implantitis. However, the clinical results following CNS treatment using PCM as observed in the present study seem to be within the range of the above-mentioned clinical improvements following treatment using PC (Karring et al. 2005, Schwarz et al. 2005a). Until now, local antimicrobial treatment of peri-implantitis has only been assessed clinically using a combination of mechanical cleansing and local application of tetracycline fibres (Mombelli et al. 2001). The authors concluded that therapy of peri-implantitis by local delivery of tetracycline had a positive effect on clinical and microbiological parameters, as significant BOP and PD reductions were achieved and maintained over a period of 12 months. When interpreting these results, however, it must be kept in mind that PD reductions at implants may predominantly reflect changes in soft tissue inflammation rather than real gain of supporting tissues. Indeed, while at healthy and mucositis sites, the probe penetration tented to stop at the histological level of connective tissue adhesion, it reached the base of the inflammatory lesion at peri-implantitis sites (Lang et al. 1992, Schou et al. 2002). This is also in agreement with the present observation that clinical assessment of PD tended to pass beyond the histological level of aJE, particularly at implant sites exhibiting higher mean values of PM–aICT. Furthermore, it must also be emphasized that radiological examination identified BD and CBI close to the histological level. Accordingly, it might be concluded that from a clinical point of view, the combined assessment of both clinical and radiological parameters seems to be appropriate for the diagnosis of peri-implant infections, even though real BIC can merely be observed histologically. The results of the present study have also demonstrated that healing appeared to

be improved in the OS groups, particularly following treatment with ERL and PCM. Indeed, morphometrical analysis of both histological specimens and radiographs revealed considerable amounts of bone regeneration in both ERL–OS and PCM–OS groups, which appeared to be limited to the intra-bony component of the former defect borders. However, newly formed bone in direct contact with the implant surface varied considerable between groups, showing statistically significant highest values for ERL–OS. In contrast, all CNS groups as well as VUS-OS were mainly characterized by the formation of a long-junctional epithelium and a dense connective tissue zone along the instrumented implant surfaces, leading to minute amounts of new bone formation merely in the most apical part of the defects. The observation that both beneficial clinical effects and bone regeneration are not inevitably associated with new BIC is in agreement with previous experimental studies in animals (Ericsson et al. 1996, Persson et al. 1996, Schou et al. 2003a, b). When interpreting the present results, however, it must also be emphasized that new BIC following OS treatment using ERL and PCM even seemed to be within the range of previously reported data following guided bone regeneration using various types of bone grafts (Hürzeler et al. 1997, Schou et al. 2003a–c). Several factors have been discussed to explain the lack of re-osseointegration at contaminated titanium surfaces. First of all, decontamination of structured implant surfaces is difficult to achieve, as conventional treatment approaches, such as plastic curettes, sonic/ultrasonic scalers and air-powder flow have been proven to be insufficient for obtaining a complete removal and elimination of both plaque biofilms and bacteria on roughened implant surfaces (Kreisler et al. 2005, Schwarz et al. 2006). Based on these observations, it might be hypothesized that different amounts of residual plaque biofilm areas (RPB) on both CNS and OS implant surfaces might have influenced peri-implant wound healing and subsequent new BIC in respective ERL, VUS and PCM groups. Indeed, the results of a previous study investigating RPB areas on SLA surfaces following instrumentation with ERL, VUS and PC have elucidated significant differences between groups (Schwarz et al. 2005b). In particular, highest amounts of mean RPB areas (%) were observed in the PC group (61.1 ± 11.4), followed by VUS (36.8 ± 4.5) and ERL (5.8 ± 5.1). These results corroborate, to a certain extent, previous findings from a case report study evaluating the effectiveness of ERL for the removal of subgingival debris from titanium implants under clinical conditions (Schwarz et al. 2003b). This investigation was conducted on eight implants (SLA and titanium plasma flamed) of two patients, considered for explantation due to severe bone loss and inflammation. Immediately before explantation, six implants were instrumented closed with ERL ($12.7\,J/cm^2$), while two implants served as a control. In comparison with the untreated control specimens, closed instrumentation of titanium implants with this type of laser resulted in an effective removal of subgingival debris without leading to any thermal damages. However, all samples of the test group revealed conspicuous amounts of residual debris (Schwarz et al. 2003b). All these data, taken together with the results of the present study seem to indicate that CNS treatment of peri-implantitis may result in higher amounts of RPB areas than OS treatment. In this context, however, it must also be taken into account that all implants of the respective OS groups were prematurely exposed to the oral environment, resulting in a new deposition of subgingival calculus. Even though histological analysis revealed a correlation between newly formed subgingival calculus and the presence of inflammatory cells in the adjacent connective tissue zone at OS implants, it is impossible to estimate to what extent bacterial recolonization of the peri-implant wound area might have influenced bone regeneration and subsequently establishment of new BIC. Another explanation for the lack of re-osseointegration may be due to the fact that it is still unknown to what extent an implant surface previously exposed to bacterial plaque biofilm formation may serve as a sufficient base to establish new BIC following decontamination. 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 & Meyer 1988, Sennerby & Lekholm 1993). Indeed, the elemental composition of unused commercially pure titanium foils was 9% titanium (Ti), 48% carbon (C), 40% oxygen (O) and traces of 10% nitrogen (N) and chlorine, whereas intra-orally contaminated foils exhibited 70% C, 20% O, 10% N and only traces of Ti ($<1\%$) (Mouhyi et al. 2000). In this context, it must be pointed out that even though instrumentation using ERL resulted in statistically significant lowest RPB areas, this kind of treatment failed to re-establish the biocompatibility of previously contaminated titanium surfaces (Schwarz et al. 2005b). However, a possible explanation for the statistically significant higher amounts of bone regeneration and new BIC in the OS groups may be due to a temporary protection of the peri-implant wound area against pathogenic bacteria residing in the oral cavity. Until now, the effects of laser-assisted decontamination on healing of peri-implantitis lesions have only been investigated for the carbon dioxide laser (CO_2) (Deppe et al. 2001, Persson et al. 2004, Stübinger et al. 2005). However, these studies reported controversial results. In particular, Persson et al. (2004) have shown that the use of a CO_2 laser in combination with hydrogen peroxide during surgical therapy of ligature-induced peri-implantitis in dogs resulted in comparable effects on bone formation and re-osseointegration as cotton pellets soaked in saline. In contrast, 16 weeks following CO_2 laser-assisted treatment, Deppe et al. (2001) reported a mean amount of newly formed bone in direct contact with the implant surface of 1.0 ± 0.52 mm for submerged implants, and 1.18 ± 0.81 mm for membrane-covered implants. However, dehisced implants merely revealed 0.38 ± 0.49 mm re-apposition of regenerated bone. Similar results were also reported by Stübinger et al. (2005), as CO_2 laser decontaminated and submerged implants revealed a mean re-apposition of regenerated bone of 1.58 ± 1.62 mm after 12 weeks of healing. All these data, taken together with the results of the present study, seem to indicate that both ERL and CO_2 lasers may be equally used for decontamination of titanium surfaces. In this context, however, it must also be emphasized that a combination of ERL, VUS and PCM with regenerative treatment procedures (i.e. barrier membranes used alone or in combination with autogenous bone or bone graft substitutes) might improve

new BIC additionally, even though the amount of re-osseointegration following guided bone regeneration is still controversially discussed (Hürzeler et al. 1997, Persson et al. 1999, Wetzel et al. 1999, Schou et al. 2003a–c).

Within the limits of the present study, it was concluded that (i) OS improved the outcome of treatment in comparison to CNS and (ii) ERL seemed to be more suitable to promote re-osseointegration at contaminated implant surfaces than PCM and VUS.

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Clinical Relevance

Scientific rationale for the study: Re-osseointegration might be influenced by both the method of surface decontamination and the mode of healing (i.e. closed treatment+non-submerged healing CNS, or open treatment+submerged healing OS).

Principal findings: The present results have indicated that OS treatment of ligature-induced peri-implantitis lesions in dogs resulted in statistically significant higher amounts of new bone-to-implant contact (BIC) than CNS treatment. However, implant surface decontamination using an Er:YAG laser (ERL) resulted in statistically significant higher BIC values than plastic curettes+local application of metronidazole gel or an ultrasonic device.

Practical implications: OS treatment of peri-implantitis using ERL seemed to be most suitable to promote re-osseointegration at contaminated implant surfaces.