Carbon Dioxide Laser and Hydrogen Peroxide Conditioning in the Treatment of Periimplantitis: An Experimental Study in the Dog

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ABSTRACT

Background: Various methods have been applied for the treatment of periimplantitis lesions. It has been reported that the procedures used have been effective in eliminating the inflammatory lesion but that re-osseointegration to the once-contaminated implant surface has been difficult or impossible to achieve.

Purpose: The aim of this study was to examine the use of carbon dioxide (CO_2) laser in combination with hydrogen peroxide in the treatment of experimentally induced periimplantitis lesions.

Materials and Methods: Three dental implants (ITI Dental Implant System®, Straumann AG, Waldenburg, Switzerland) were placed in each side of the edentulous mandible of four beagle dogs. Implants with a turned surface and implants with a sand-blasted large-grit acid-etched (SLA) surface (SLA®, Straumann AG, Waldenburg, Switzerland) were used. Experimental periimplantitis was induced during 3 months. Five weeks later each animal received tablets of amoxicillin and metronidazole for a period of 17 days. Three days after the start of the antibiotic treatment, full-thickness flaps were elevated, and the granulation tissue in the bone craters was removed. In the two anterior implant sites in both sides of the mandible, a combination of CO_2 laser therapy and application of a water solution of hydrogen peroxide was used. The implant in the posterior site of each quadrant was cleaned with cotton pellets soaked in saline. Biopsy specimens were obtained 6 months later.

Results: The amount of re-osseointegration was 21% and 82% at laser-treated turned-surface implants and SLA implants, respectively, and 22% and 84% at saline-treated turned-surface implants and SLA implants, respectively.

Conclusions: The present study demonstrated the following: (1) a combination of systemic antibiotics and local curettage and débridement resulted in the resolution of experimentally induced periimplantitis lesions; (2) at implants with a turned surface, a small amount of re-osseointegration was observed at the base of the bone defects whereas a considerable amount of re-osseointegration occurred at implants with an SLA surface; and (3) the use of CO_2 laser and hydrogen peroxide during surgical therapy had no apparent effect on bone formation and re-osseointegration.

KEY WORDS: antibiotics, biopsy, dental implants, dogs, histology, surgery, titanium

Various methods have been applied for the treatment of periimplantitis lesions.[1–6] It has been reported that the procedures used have been effective in eliminating the inflammatory lesion but that re-osseointegration to the once-contaminated implant surface has been difficult or impossible to achieve. In a recent experimental study on treatment of periimplantitis lesions, it was suggested that ''neither the chemical nor the mechanical cleaning procedures hitherto used may recondition the once contaminated implant surface to an extent that will allow re-osseointegration to occur.''[7]

Limited information is available in regard to the surface characteristics of contaminated implants. Mouhyi and colleagues[8] examined failed Brånemark

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System® implants (Nobel Biocare AB, Göteborg, Sweden) with scanning electron microscopy (SEM) and x-ray–induced photoelectron spectroscopy (XPS). The authors claimed that the implant harbored debris and contaminants that included remnants of blood, fibers, and microorganisms. The XPS analyses revealed that the surface layer of contaminated implants contained small amounts of titanium and were about 75% carbon, 18% oxygen, and 4% nitrogen whereas uncontaminated implant surfaces were 17% titanium, 25% carbon, 55% oxygen, and 3% nitrogen.

Different techniques for cleaning contaminated titanium surfaces and for reinstituting a surface layer that is comparable to a pristine implant surface have been evaluated. The effect of applying carbon dioxide (CO_2) laser and hydrogen peroxide solution to a contaminated titanium surface was examined in vitro by Mouhyi and colleagues.[9] They attached titanium foils to the dentures of two patients. The foils were removed after 24 hours, placed in a bacterial culture solution, and incubated at 37°C for 24 hours. Different cleaning procedures were tested, including rinsing with citric acid, hydrogen peroxide solutions, and water, followed by the application of CO_2 laser. The foils were examined with SEM and XPS techniques. It was reported that treatment with CO_2 laser and hydrogen peroxide solutions resulted in a surface containing low levels of titanium and oxygen but high levels of carbon in comparison to pristine titanium foils. The aim of the present experiment was to further study the use of CO_2 laser in combination with hydrogen peroxide in the treatment of experimentally induced periimplantitis lesions.

MATERIALS AND METHODS

The outline of the experiment (Figure 1) has been previously described.[10] In brief, three solid-screw 8.0 × 3.3 mm dental implants (ITI Dental Implant System®, Straumann AG, Waldenburg, Switzerland) were placed in each side of the edentulous ridge of the mandibles of four beagle dogs. Implants used in the left side were designed with a turned surface whereas implants with a sand-blasted large-grit acid-etched (SLA) surface were used in the right side. The implants were installed in such a way that the borderline between the intraosseus and transmucosal part of the implant coincided with the bone crest. Some surface characteristics of the two types of implants used in the present study were recently reported on.[11] The Sa values were 0.35 ± 0.17 μm for the turned-surface implants and 2.29 ± 0.59 μm for the SLA implants.

After implant installation a plaque control program including tooth and implant cleaning twice per week was initiated. Experimental periimplantitis was induced 3 months after implant installation by placing cotton ligatures in a submarginal position around the implants and allowing plaque formation.[12] Three months later, when radiographic examination of the implant sites disclosed that approximately 50% of the initial bone support had been lost, the ligatures were removed.

An antibiotic regimen was initiated 1 month later. Each animal received amoxicillin (Imacillin®

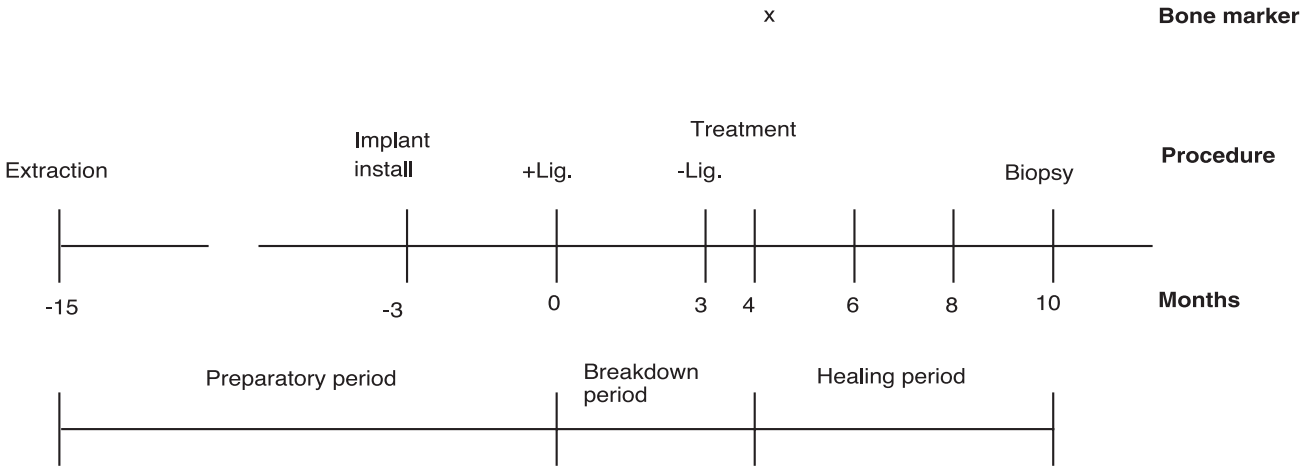

Figure 1 Design of the experiment. Ligatures were placed around the implants at 0 months (+ lig.) and were removed at 3 months (− lig.). Treatment was performed at 4 months, and a bone marker was injected at 4.5 months. Biopsies were performed at 10 months.

[AstraZeneca, Södertälje, Sweden], 250 mg twice per day) and metronidazole (Elyzol® [Dumex, Copenhagen, Denmark], 250 mg twice per day) tablets for 17 days.

Three days after the start of the antibiotic regimen, buccal and lingual full-thickness flaps were elevated. The granulation tissue in the bone craters around the implants was removed with curettes, and the exposed implant surfaces were treated in the following manner: In the two anterior implant sites in both sides of the mandible, a combination of laser therapy and the application of a 10 mM water solution of hydrogen peroxide was used. The CO_2 laser unit (Lasersat 20™, Satelec, Mèrignac, France) had the following settings: power, 8 W; pulse width, 10 ms; frequency, 20 Hz; and irradiation cycle, 5 seconds. The generated wavelength of the CO_2 laser was 10.6 μm, and the beam diameter was 300 nm at the focal point located 12 mm in front of the probe. The CO_2 laser was applied during continuous irrigation with a 100 mM solution of hydrogen peroxide (Figure 2). The implant in the posterior site of each quadrant was carefully cleaned with cotton pellets soaked in saline. The mucoperiosteal flaps were repositioned to cover all implants and were closed with mattress and interrupted sutures.

Two weeks after surgery the sutures were removed, and a fluorochrome (oxytetracycline [Oxysentin®, Ciba-Geigy AG, Basel, Switzerland], 16 mg/kg of body weight) was injected intravenously. The fluorochrome administration was repeated 24 hours later. Three weeks after suture removal, the marginal portion of all implants had penetrated the mucosa, and a plaque control program consisting of tooth and implant cleaning was initiated.

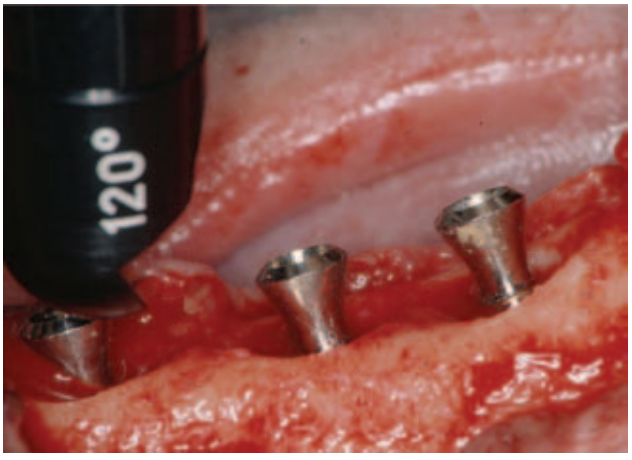

Figure 2 Clinical photograph of implant sites in the left side (turned implants) when the flap has been elevated. The head of the laser instrument is seen to the left.

Six months after the surgical therapy (month 10; see Figure 1), the animals were sacrificed with an overdose of Pentothal® Natrium (Abbot Laboratories, Chicago, IL, USA) and perfused with a fixative[13] through the carotid arteries.

The mandibles were removed, and block biopsy specimens of each experimental site were dissected. The biopsy specimens were prepared for ground sectioning according to methods described by Donath and Breuner.[14] The blocks were first divided in a mesiodistal direction with a cutting grinding unit (EXAKT®, EXACT Apparatebau GmbH & Co., Norderstedt, Germany). From each buccal and lingual portion, one mesiodistal section was prepared. The remaining buccal and lingual portions of the blocks were cut in a buccolingual direction. All sections were reduced to a thickness of about 20 μm. From each experimental site, therefore, six sections representing the mesial, distal, buccal, and lingual aspects of the implant were produced.

The histologic examination was performed with a Leica DM-RBE® microscope (Leica Mikroskopie und Systeme GmbH, Wetzlar, Germany) equipped with an image analysis system (QWin, Leica Microsystems AG, Wetzlar, Germany). The unstained sections were examined under fluorescence light and with a filter that was compatible with the fluorochrome. The location of the fluorochrome in the sections indicated the base and the lateral wall of the infrabony defect immediately after treatment (Figures 3B and 4B). The base of the defect (BD) was identified, and the size of the defect area was assessed.

The sections were stained in toluidine blue. The following landmarks were identified and used for the linear measurements (Figure 5): the implant shoulder (IC), the marginal level of bone in contact with the implant (CBI), the marginal portion of the periimplant mucosa (PM), the apical termination of the barrier epithelium (aJE), and the marginal position of the lateral bone wall of the defect (BC).

The bone tissue was further examined by use of a morphometric technique. A lattice composed of 100 points (× 200 magnification) was superimposed (1) over the regenerated bone in the previous defect and (2) in a region occupied by "old bone" in the apical part of the implant. The proportions occupied by lamellar bone, bone marrow, and woven bone were

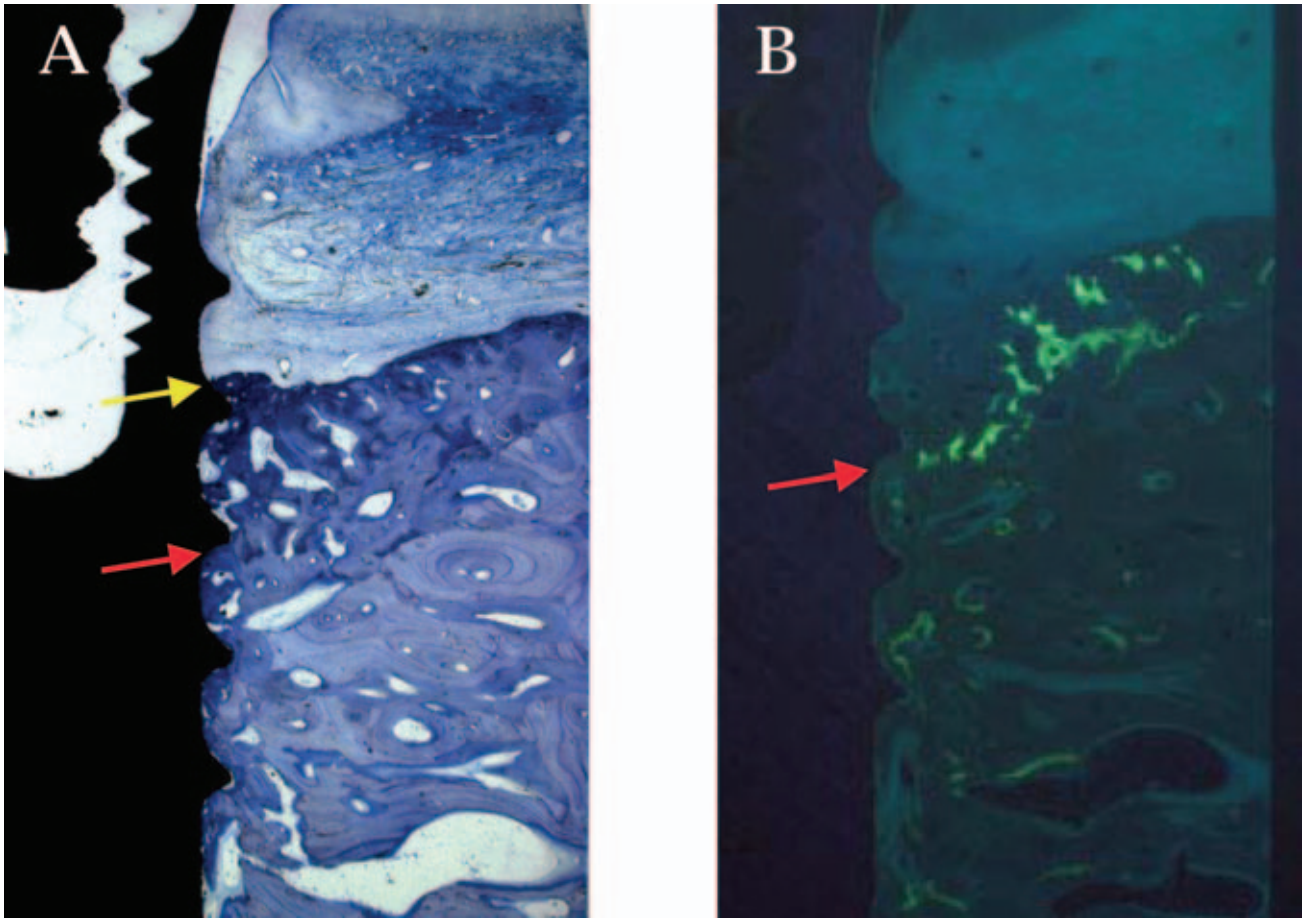

Figure 3 Mesiodistal ground section of an implant with the adjacent periimplant tissues. *A*, Light microscopic photo of an implant with a sand-blasted large-grit acid-etched surface. *Yellow arrow* indicates the most coronal bone in contact with the implant (CBI); *red arrow* indicates the base of the defect (BD), identified by the position of the bone marker in Figure 3B. Notice the direct contact between the newly formed bone and the implant surface (× 25 original magnification; stained with toluidine blue). *B*, Photograph made with fluorescent light. Bone marker (oxytetracycline) seen as yellow areas identifies the apical and lateral borders of the produced bone defect (*red arrow*) (× 25 original magnification).

assessed. The percentage of bone-to-implant contact was determined within the ''regenerated'' area.

Mean values and standard deviations for the different variables were calculated for each implant unit and animal.

RESULTS

Histologic Examination and Measurements

New bone formation had occurred in the infrabony defects of all experimental sites. In sections from sites including the SLA implants, the newly formed bone was in direct contact with the implant surface (see Figure 3A). This re-osseointegration occurred at both laser- and saline-treated surfaces. In sites harboring implants with turned surfaces, however, a 50 to 200 μm wide zone of connective tissue was consistently found to be present between the newly formed bone and the titanium surface (see Figure 4A).

The histometric measurements are presented in Table 1. The distance between the soft tissue margin and the implant shoulder (PM to IC) varied from 1.19 to 1.47 mm. Although the dimension of the connective-tissue interface (aJE to CBI) was similar at all laser- and saline-treated sites, the barrier epithelium (PM to aJE) appeared to be longer at sites exposed to laser therapy than at saline-treated sites (2.18 mm vs 1.87 mm at turned implants and 2.31 mm vs 1.87 mm at SLA implants).

The depth of the infrabony defect (BC to BD) that was assessed with the fluorochrome marker as reference was 2.03 to 2.12 mm at the turned implants and 1.45 to 1.72 mm at the SLA implants. At the sites with turned implants, laser therapy and saline therapy resulted in

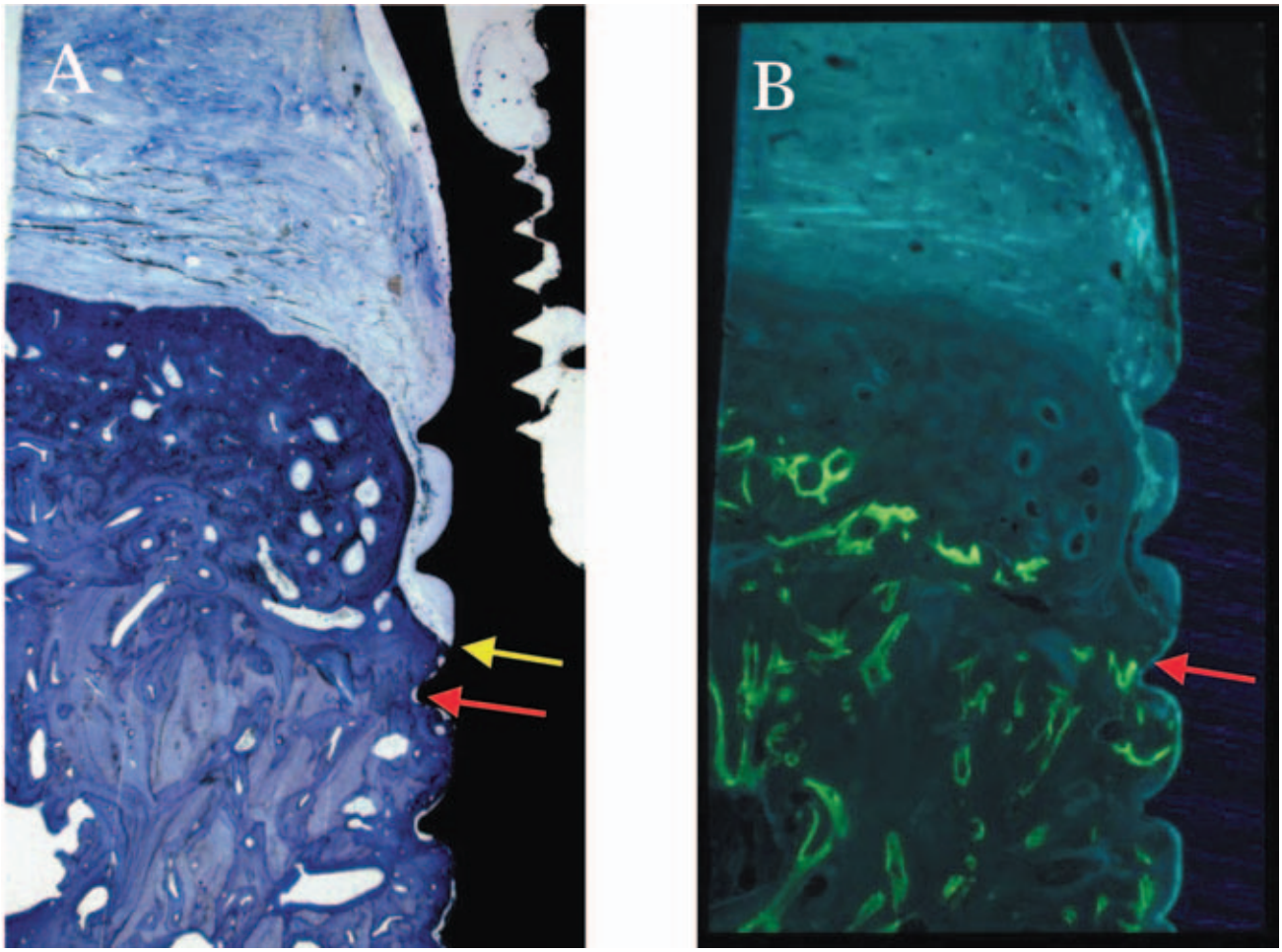

Figure 4 Mesiodistal ground section of an implant with the adjacent periimplant tissues. *A*, Light microscopic photo of an implant with a turned surface. *Yellow arrow* indicates the most coronal bone in contact with the implant (CBI); *red arrow* indicates the base of the defect (BD), identified by the position of the bone marker in photo 4B. Notice connective tissue separating the bone from the implant surface (× 25 original magnification; stained with toluidine blue). *B*, Photograph made with fluorescent light. Bone marker (oxytetracycline) seen as yellow areas identifies the apical and lateral borders of the produced bone defect (*red arrow*) (× 25 original magnification).

re-osseointegration of 0.46 mm (21%) and 0.42 mm (22%), respectively. The corresponding figures for the sites with SLA implants were 1.13 mm (74%) for laser therapy and 1.22 mm (84%) for saline therapy.

The size of the defect that developed during the ligature breakdown period varied between 4.20 and 5.25 mm^2. The amount of bone fill that occurred following the different treatment procedures varied from 3.18 to 3.98 mm^2. This amount of bone fill corresponded to 72 to 82% of the preexisting defect area.

The morphometric measurements are presented in Table 2. On average the regenerated bone was 44.5 to 50.5% lamellar bone, 37.5 to 45.8% woven bone, and 6.8 to 12.4% bone marrow. The corresponding figures describing the ''old bone'' lateral to the defect area were 85.8 to 89.1% lamellar bone and 10.9 to 14.2% bone marrow.

For laser-treated sites the percentage of bone-to-implant contact (Table 3) within the ''re-osseointegration zone'' was 59.3% at turned implants and 72.1% at SLA implants. At the saline-treated sites the corresponding respective percentages were 67.6% and 62.9%.

DISCUSSION

The present study demonstrated that a combination of systemic antibiotics and local curettage and débridement resulted in the resolution of experimentally induced periimplantitis lesions. At implants with a turned surface, a small amount of re-osseointegration was observed at the base of the bone defects whereas a considerable amount of re-osseointegration had occurred at implants with an SLA surface. Further, the use of CO_2 laser and hydrogen peroxide during

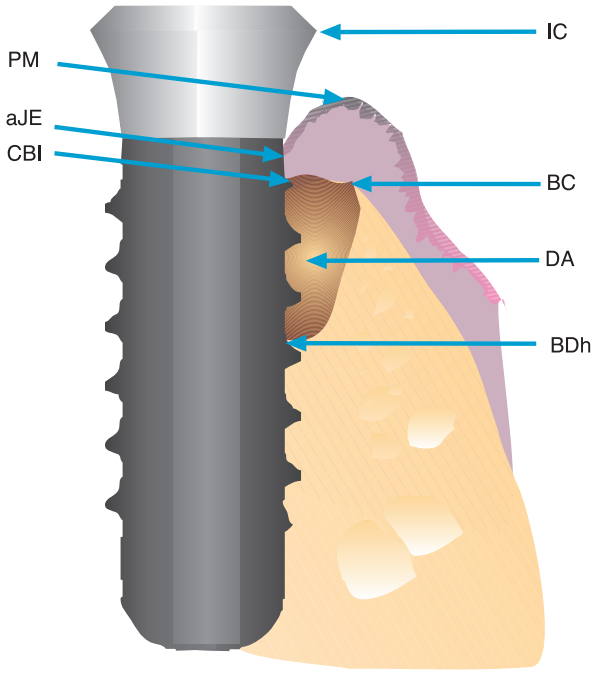

Figure 5 Schematic drawing illustrating the landmarks used for the histometric measurements. (aJE = level of the apical termination of the barrier epithelium; BC = level of the lateral bone wall of the defect; BDh = base of the defect; CBI = marginal level of bone in contact with the implant; DA = defect area; IC = implant shoulder; PM = marginal portion of the periimplant mucosa).

surgical therapy had no apparent effect on bone formation and re-osseointegration.

The effect of the application of a CO_2 laser and hydrogen peroxide solution to a contaminated titanium surface was examined in vitro by Mouhyi and colleagues,[9] who tested different cleaning procedures, including rinsing with citric acid, hydrogen peroxide solutions, and water, followed by the application of CO_2 laser on titanium foils. They reported that the treatment with CO_2 laser and hydrogen peroxide solutions resulted in a surface containing low levels of titanium and oxygen but high levels of carbon, in comparison to pristine titanium foils. Mouhyi and colleagues[9] also reported that the best results were obtained when a combination of laser, hydrogen peroxide, and citric acid was used. The results from the present experiment showed that a contaminated surface does not prevent re-osseointegration, at least not at implants with a rough surface. This observation is in agreement with findings reported by Deppe and colleagues.[15] They placed implants with a plasma-sprayed surface in the mandibles of beagle dogs. Experimental periimplantitis that resulted in the loss of 30 to 50% of the periimplant bone was induced, and three different treatment procedures were applied. The implants in one group were decontaminated with an air-powder abrasive unit while the implants in a second group were treated with a CO_2 laser unit (continuous wave, 2.5 W, and duration of 12 × 5 seconds). In the third group of implants, a combination of the air-powder treatment and laser treatment was applied. Deppe and colleagues[15] reported that after a healing period of 4 months, newly formed bone was found to be in contact with the implants in all treatment groups. Referring to an

Table 1 Results of Histometric Measurements at Laser-Treated and Saline-Treated Implants: Mean Values and Standard Deviations*

	Turned Implants		SLA Implants	
	Laser	Saline	Laser	Saline
IC–PM (mm)	1.19 (0.34)	1.33 (0.21)	1.47 (0.26)	1.31 (0.45)
PM–aJE (mm)	2.18 (0.65)	1.87 (0.35)	2.31 (0.37)	1.71 (0.46)
aJE–CBI (mm)	1.95 (0.15)	1.90 (0.39)	1.80 (1.09)	1.62 (0.62)
BC–BD (mm)	2.12 (0.35)	2.03 (0.52)	1.77 (0.82)	1.45 (0.33)
Re-osseointegration (mm)	0.46 (0.14)	0.42 (0.13)	1.13 (0.32)	1.22 (0.35)
Re-osseointegration (% of bone defect)	21 (9.0)	22 (16.7)	74 (30.0)	84 (8.6)
Defect area (mm^2)	5.05 (1.81)	5.34 (1.19)	5.14 (2.68)	4.20 (0.93)
Bone fill (mm^2)	3.54 (1.37)	3.81 (0.84)	3.98 (1.53)	3.18 (0.57)
Bone fill (%)	75.0 (6.7)	72.0 (3.9)	82 (12.2)	76.0 (10.1)

SLA = sand-blasted large-grit acid-etched.
*Values in parentheses represent standard deviations.

Table 2 Results of Morphometric Measurements of Periimplant Bone Tissue at Laser-Treated and Saline-Treated Implants: Mean Values and Standard Deviations*

	Turned Implants		SLA Implants	
	Laser	Saline	Laser	Saline
Regenerated bone (%)				
Lamellar bone	48.3 (7.2)	50.5 (14.6)	44.5 (14.1)	50.1 (7.4)
Bone marrow	11.1 (5.6)	6.8 (4.3)	9.6 (4.6)	12.4 (3.7)
Woven bone	40.4 (11.4)	42.6 (13.4)	45.8 (14.9)	37.5 (5.1)
"Old bone" (%)				
Lamellar bone	86.3 (6.0)	89.1 (3.5)	86.6 (4.8)	85.8 (8.2)
Bone marrow	13.7 (5.4)	10.9 (3.5)	13.4 (4.9)	14.2 (6.2)
Woven bone	0	0	0	0

SLA = sand-blasted large-grit acid-etched.
*Values in parentheses represent standard deviations.

experimental study in dogs, Kolonidis and colleagues[16] reported that osseointegration can occur on implant surfaces that initially were exposed to plaque and were subsequently cleaned. In the study referred to, implants with a turned surface were partially installed, and plaque was allowed to accumulate on the exposed implant surfaces for 5 weeks. Following cleaning with either citric acid or hydrogen peroxide, the implants were retrieved and were reinstalled in newly prepared sites on the contralateral side of the mandible. After healing, histologic evaluation revealed that all treated implant surfaces were associated with direct bone-to-implant contact. This finding is in contrast to reported results from the treatment of experimental periimplantitis on implants with a turned surface.[3] In this context it is important to note that no defects were present around the implants in the study by Kolonidis and colleagues[16] whereas crater-formed defects are present in experimental periimplantitis models.[3]

Table 3 Percentage of Bone-to-Implant Contact within the Zone of Re-osseointegration at Laser-Treated and Saline-Treated Implants: Mean Values and Standard Deviations*

Turned Implants		SLA Implants	
Laser	Saline	Laser	Saline
59.3 (8.4)	67.6 (10.3)	72.1 (12.4)	62.9 (2.1)

SLA = sand-blasted large-grit acid-etched.
*Values in parentheses represent standard deviations.

In the present study, implant surfaces were subjected to the combination of CO_2 laser and hydrogen peroxide in an attempt to improve conditions for re-osseointegration. It has been suggested that CO_2 laser has the ability to sterilize a surface by a temperature increase that leads to the destruction of cells and microorganisms.[17] The use of CO_2 laser together with the application of hydrogen peroxide was based on the theory that the oxide layer can be restored by an increase in temperature and by the presence of an oxidant.[18] Mouhyi and colleagues[19] studied the soft tissue response to titanium surfaces that were cleaned with the combined use of hydrogen peroxide and CO_2 laser. Contaminated and subsequently cleaned titanium cover screws were placed in the abdominal walls of rats, and the thickness of a surrounding fibrous capsule and the presence of macrophages were analyzed. It was reported that the combined CO_2 laser and hydrogen peroxide decontamination procedure resulted in a significant reduction in the thickness of fibrous capsules and in the number of macrophages when compared to the untreated contaminated controls.

The current study showed that re-osseointegration occurred only to a small extent at implants with a turned surface whereas a high degree of re-osseointegration was observed at the SLA implants. No differences regarding re-osseointegration were detected between laser-treated and saline-treated sites in any of the implant groups. The apparent lack of effect of the combined application of CO_2 laser and hydrogen peroxide in the present study may thus indicate that

surface characteristics are of more importance for re-osseointegration than is a possible ''decontamination'' effect from laser therapy.

One of the problems related to different treatment procedures for contaminated implant surfaces is the risk of tissue damage caused by an increase in temperature. Eriksson and Albrektsson[20] studied the influence of temperature increase on bone tissue in rabbits. They reported that a relatively small increase in temperature from 47°C to 50°C during the course of 1 minute resulted in bone tissue damage. In an in vitro study, however, Mouhyi and colleagues[21] found that the temperature increase during surface decontamination with a CO_2 laser unit is small and that there is a low risk of tissue damage. The observation in the present study that an implant site treated by CO_2 laser in combination with a hydrogen peroxide solution healed with a high degree of re-osseointegration at SLA implants may support such a conclusion.

CONCLUSIONS

The present study demonstrated the following: (1) a combination of systemic antibiotics and local curettage and débridement resulted in the resolution of experimentally induced periimplantitis lesions; (2) at implants with a turned surface, a small amount of re-osseointegration was observed at the base of the bone defects whereas a considerable amount of re-osseointegration occurred at implants with an SLA surface; and (3) the use of CO_2 laser and hydrogen peroxide during surgical therapy had no apparent effect on bone formation and re-osseointegration.

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