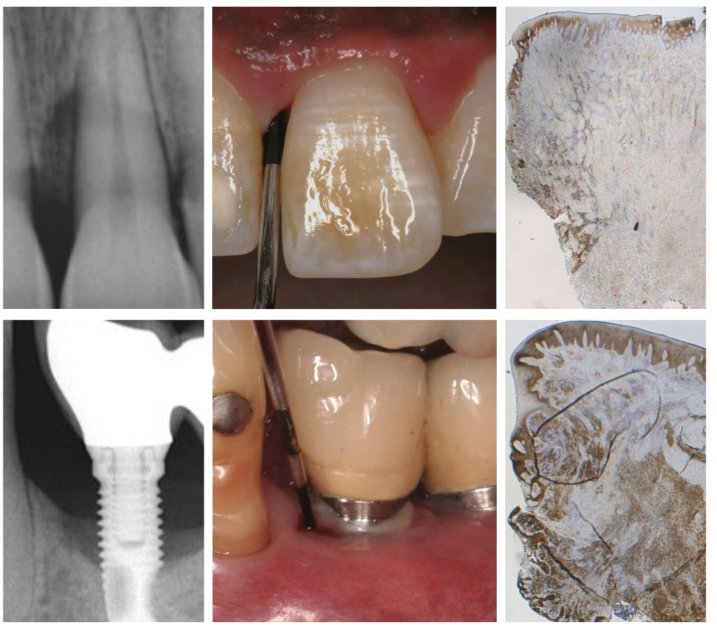
The Sahlgrenska Academy

Peri-implantitis and Periodontitis

Experimental and Clinical Studies



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Cover illustration: radiographs, clinical images and histological sections (immunohistochemical marker CD138) representing human periodontitis and peri-implantitis.

To Madeleine and Noah

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Abstract

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Peri-implantitis is an increasing problem in implant dentistry. The current series of studies employed a translational approach with the aim to compare peri-implantitis and periodontitis lesions and evaluate the influence of implant surface characteristics and the adjunctive use of systemic antibiotics/local antiseptics on healing following surgical treatment of peri-implantitis.

Tissue reactions following ligature removal in experimental periodontitis and peri-implantitis were analyzed in a dog model (**Study I**). Histopathological characteristics in human peri-implantitis and periodontitis lesions were evaluated in 80 patients (**Study II**). Labrador dogs were used to analyze the effect of surgical treatment of experimental peri-implantitis at implants with different surface characteristics using different anti-infective procedures (**Study III**). 100 patients with severe peri-implantitis were treated surgically with or without adjunctive systemic antibiotics or the local use of chlorhexidine for implant surface decontamination. Treatment outcomes were evaluated after 1 year. A binary logistic regression analysis was performed to identify factors influencing the probability of treatment success (**Study IV**).

It was demonstrated that :

- the amount of bone loss that occurred during the period following ligature removal was significantly larger at implants with a modified surface than at implants with a non-modified surface and at teeth. The histological analysis revealed that peri-implantitis sites exhibited inflammatory cell infiltrates that were larger, extended closer to the bone crest and contained larger proportions of neutrophil granulocytes and osteoclasts than in periodontitis. (**Study I**)
- peri-implantitis lesions were more than twice as large and contained significantly larger area proportions, numbers, and densities of CD138-, CD68-, and MPO-positive cells than periodontitis lesions. (**Study II**)
- the local use of chlorhexidine has minor influence on resolution of peri-implantitis following surgical treatment. (**Study III**)
- treatment outcome was influenced by implant surface characteristics. (**Study III** and **IV**)
- the adjunctive use of systemic antibiotics increased the probability for treatment success at implants with modified surfaces but not at implants with a non-modified surface. (**Study IV**)

Preface

The present thesis is based on the following publications, which will be referred to in the text by their Roman numerals.

I. **Carcuac O.**, Abrahamsson I., Albouy JP., Linder E., Larsson L., Berglundh T. (2013) Experimental periodontitis and peri-implantitis in dogs. *Clinical Oral Implant Research* **24**, 363-371

II. **Carcuac O.**, Berglundh T. (2014) Composition of human periodontitis and peri-implantitis lesions. *Journal of Dental Research* **93(11)**, 1083-1088

III. **Carcuac O.**, Abrahamsson I., Charalampakis G., Berglundh T. (2015) The effect of the local use of chlorhexidine in surgical treatment of experimental peri-implantitis in dogs. *Journal of Clinical Periodontology* doi: 10.1111/jcpe.12332 [Epub ahead of print]

IV. **Carcuac O.**, Derks J., Charalampakis G., Abrahamsson I., Wennström JL., Berglundh T. (2015) Adjunctive systemic antibiotics enhance treatment outcomes of surgical therapy of peri-implantitis at implants with modified surface but not at implants with non-modified surfaces. A randomized controlled clinical trial. *In manuscript.*

List of abbreviations

Common abbreviations used in this thesis are listed according to their first appearance.

ICT	Inflamed connective tissue
PMN	Polymorphonuclear cell
IL-1	Interleukine 1
IL-6	Interleukine 6
TNF-α	Tumor necrosis factor- alpha
IL-8	Interleukine 8
PIM	Peri-implant mucosa
CT	Connective tissue
PE	Pocket epithelium
PI	Peri-implantitis
AG	Aggressive periodontitis
CP	Chronic periodontitis
PPD	Probing pocket depth
BoP	Bleeding on probing
IHC	Immunohistochemical
CAL	Clinical attachment loss
e-PTFE	Expanded polytetrafluoroethylene
SLA	Sandblasted large acid-etched
TPS	Titanium plasma sprayed
Er-YAG	Erbium doped yttrium-aluminium-granet
Dnr	Diarienumber
NP	Narrow platform
S.D.	Standard deviation
SoP	Suppuration on probing
AB	Systemic antibiotics
AS	Local antiseptics
CVD	Cardiovascular disease
GM/PM	Gingival/peri-implant mucosa margin
A/F	Abutment/fixture junction
CEJ	Cemento-enamel junction
aPlaque	Apical termination of the biofilm
aPE	Apical termination of the pocket epithelium
B	Marginal bone level closest to tooth/implant
BC	Most coronal extension of the bone crest
cICT	Coronal extension of the ICT
aICT	Apical extension of the ICT
Bw	Lateral bone wall of the intra-bony defect
AGNB	Aerobie gram negative bacilli
MPO	Myeloperoxydase
IgG	Immunoglobuline G
TVC	Total viable count
OR	Odds ratio

Introduction

Peri-implantitis is defined as inflammation in peri-implant soft tissues and associated loss of supporting bone (Lindhe & Meyle, 2008). Several reviews have tried to assess the prevalence of peri-implantitis (Zitzmann & Berglundh, 2008; Mombelli et al., 2012; Derks & Tomasi, 2014) and data from cross-sectional studies of different patient groups (Fransson et al., 2005; 2008; Ferreira et al., 2006; Roos Jansåker et al., 2006; Koldsland et al., 2010; Zetterqvist et al., 2010; Dvorak et al., 2011; Mir-Mari et al., 2012; Casado et al., 2013; Marrone et al., 2013; Cecchinato et al., 2013, 2014) revealed that the prevalence of peri-implantitis ranged from 1 % to 47 %. Tomasi & Derks (2012) addressed the complexity of case definitions in the literature, which, may explain the large variation in prevalence of peri-implant diseases reported in different studies. Such a limitation together with varying time of follow-up were considered in a systematic review by Derks & Tomasi (2014). Meta-analysis revealed an estimated weighted mean prevalence for peri-implantitis of 22 % (95 % CI: 14 %-30 %).

Peri-implantitis and periodontitis lesions

Although clinical and radiological signs of periodontitis and peri-implantitis have many features in common, results from pre-clinical *in vivo* studies indicate that significant histopathological differences exist, which may explain differences in disease onset and progression (Lindhe et al., 1992; Schou et al., 1993; Berglundh et al., 2011). In a review on periodontitis and peri-implantitis lesions, Berglundh et al. (2011) appraised information on the different lesions. The authors reported that few pre-clinical *in vivo* studies comparing experimental ligature-induced peri-implantitis and periodontitis lesions in animals were available (Table 1) and that studies including structured comparisons between human peri-implantitis and periodontitis lesions were lacking (Table 2).

Pre-clinical *in vivo* studies in animals

Most experimental studies on peri-implantitis used the ligature-model to induce breakdown of peri-implant soft and hard tissues. This model was extensively used in studies on experimental periodontitis and was introduced to promote rapid tissue breakdown as opposed to earlier studies on the natural development of periodontitis in dogs with attachment and bone loss occurring after several years (Lindhe et al., 1973, 1975; Hamp & Lindberg, 1977). Thus, ligatures were used together with plaque formation in order to initiate and maintain a pathological process in gingival tissues. Placement of a ligature in a subgingival position disrupts the soft tissue seal around teeth and implants and opens the pocket for biofilm accumulation. While a ligature made of cotton or silk may not induce bone loss by itself, the developing inflammatory process in the connective tissue that results

from biofilm formation mediates tissue destruction during the experiment. The early response to ligature placement and biofilm accumulation in experimental periodontitis was described in a study in monkeys (Heijl et al., 1976). It was observed that the rate of tissue breakdown decreased over time and that ligatures had to be removed and replaced to promote continuous tissue destruction. In most studies on experimental periodontitis, ligatures were removed about one month prior to biopsy to allow acute lesions to become chronic. Using a similar procedure in experimental peri-implantitis, results indicated that the spontaneous resolution observed in experimental periodontitis sites did not occur after ligature removal around implants (Lindhe et al., 1992). In this study, cotton ligatures were placed in a subgingival position around teeth and implants in five beagle dogs and plaque was allowed to accumulate. While the ligatures were removed after 6 weeks, plaque formation continued and after an additional 4-week period clinical and radiological examinations were performed and block biopsies were obtained. It was reported that clinical signs of inflammation and radiographic bone loss was more pronounced in peri-implantitis than in periodontitis sites. In addition, the histological examination revealed that the inflamed connective tissue (ICT) was larger at implants than at teeth. It was observed that peri-implantitis lesions extended to the bone crest, while the periodontitis lesions were consistently separated from the bone crest by a zone of non inflamed connective tissue. Similar findings were presented by Schou et al. (1993) studying experimental peri-implantitis and periodontitis in monkeys. It was reported that bone loss was more pronounced around implants than at teeth and that bone loss was associated with a high number of osteoclasts in the histological specimens.

A new approach to the ligature-model was introduced by Zitzmann et al. (2004). Ligatures were placed in a submarginal position around Brånemark implants in 5 Labrador dogs. The combination of the local trauma elicited by the ligatures and concomitant plaque accumulation resulted in bone defects and clinical signs of inflammation around all implants. The ligatures were removed and during the subsequent 1-year period of continuous plaque formation, additional bone loss occurred around several implants. It was concluded that spontaneous progression of peri-implantitis may occur after the removal of ligatures. This model of "spontaneous progression in experimental peri-implantitis" was subsequently applied by Berglundh et al. (2007) and Albouy et al. (2008, 2009, 2012). Similar observations of a continuous destructive process following removal of ligatures have not been reported in experimental periodontitis.
Using the same ligature-model and sampling of biopsies that included the entire peri-implant and periodontal hard and soft tissue components, a pre-clinical *in vivo* model was used in **study I** to evaluate differences in tissue reactions in experimentally induced periodontitis and peri-implantitis in dogs.

Human biopsy material

As findings from experimental studies should be validated in human protocols and more comprehensive analyses of cellular and functional characteristics of the lesions are required, evaluations of human biopsies are needed. In the abovementioned review on periodontitis and peri-implantitis lesions, Berglundh et al. (2011) reported that comprehensive information on human periodontitis lesions exists, while few studies have examined peri-implantitis lesions prepared from human samples (Sanz et al., 1991; Cornelini et al., 2001; Gualini & Berglundh, 2003; Berglundh et al., 2004). In addition, the analyses of human peri-implantitis were based on small samples.

Sanz et al. (1991) analyzed soft tissue biopsies from 6 patients with peri-implantitis and reported that about 2/3 of the connective tissue portion of the biopsy was occupied by an infiltrate consisting of plasma cells, mononuclear cells and enlarged blood vessels. Similar findings were presented by Cornelini et al. (2001) in a study on biopsies prepared from 10 patients with peri-implantitis. Gualini & Berglundh (2003) examined immunohistochemical characteristics of soft tissue biopsies obtained from 16 patients and reported that peri-implantitis lesions were considerably larger and contained significantly greater proportions of B cells and elastase-positive cells than mucositis lesions. Berglundh et al. (2004) analyzed soft tissue biopsies obtained from 12 implants with severe peri-implantitis in 6 patients. The histological analysis demonstrated that lesions occupied almost the entire connective tissue compartment and extended apically of the pocket epithelium.

Comparisons between human peri-implantitis and periodontitis lesions are rare. Bullon et al. (2004) analyzed soft tissue biopsies from 5 cases with peri-implantitis and 5 patients with aggressive periodontitis. It was reported that both peri-implantitis and periodontitis lesions presented with plasma cells, macrophages and lymphocytes, among which T cells were more common than B cells. Konttinen et al. (2006) analyzed Il-1, IL-6, TNF-α in peri-implant and/or gingival samples from failing implants, chronic periodontitis and healthy gingiva and reported that cytokines with a potential to activate osteoclasts were found in both peri-implantitis and chronic periodontitis with a higher proportions of IL-1 and IL-6 in peri-implantitis than in periodontitis lesions. Venza et al. (2010) analyzed soft tissue biopsies collected from different patient-groups and reported that peri-implantitis specimens exhibited higher mRNA expression of IL-6, IL-8, and TNF-α than periodontitis samples. In a study on genome-wide transcriptome profiles in gingival specimens obtained from small patient groups with periodontitis and peri-implantitis, Becker et al. (2014) concluded that the two conditions represent distinct entities with different mRNA signatures.

Comparisons between human peri-implantitis and periodontitis lesions require sufficiently powered patient samples to unravel critical differences between the conditions. Thus, **study II** was performed to compare local host response characteristics in peri-implantitis and periodontitis in humans at the cellular level.

Table 1. Pre-clinical in-vivo studies comparing peri-implantitis and periodontitis lesions - clinical and histological analyses

References	Number of animals/implants/teeth involved	Outline of the experiment	Methods	Results
Lindhe et al. (1992)	•Five dogs. - 10 implants (Brånemark system). - 10 teeth (3rd and 4th mandibular premolars).	•6 months with plaque control after abutment connection. •Ligatures for 6 weeks at implants and contra-lateral premolars (replaced after 3 weeks). •Plaque accumulation for additional 4 weeks without ligature.	•Clinical and radiological examination of implants and teeth 1 month after ligature removal. •Biopsies from implant and tooth sites. •Histometric and morphometric measurements.	•Clinical and radiological signs of tissue destruction more pronounced at PiM than at teeth. •PE was ulcerated in PiM and tooth sites. •ICT size larger in PiM than at tooth sites ICT dominated by PMN and plasma cells in PiM, by macrophages and lymphocytes in tooth sites. ICT extended into bone marrow at implant sites while a non-infiltrated supra alveolar CT is present between ICT and alveolar bone crest at tooth sites.
Lang et al. (1993)	•Four cynomolgus monkeys. - 16 implants (ITI system : implant with titanium plasma-coated rough surfaces). - 4 teeth (3rd mandibular molar).	•60 days of healing after implant placement with plaque control 3 times a week. •Plaque accumulation for 30 days. •Ligatures for 8 months at 8 implants and all 3rd mandibular molars (replaced at 3 and 6 months).	•Clinical examination every month following ligature placement. • Radiological examination at 1, 2, 5, 6 and 8 months following ligature placement.	•Clinical and radiological signs of tissue destruction at both implants and teeth sites with similar rate of development.
Schou et al. (1993)	•Eight cynomolgus monkeys. - 16 implants (Titanium-coated cylindric polycarbonate implants). - 16 teeth (8 ankylosed maxillary molars and 8 normal maxillary pre-molars).	•3 months healing after implant placement. •Ligatures for 7 weeks at implants and teeth.	•Clinical examination at 2, 4 and 7 weeks following ligature placement. • Radiological examination at 2, 4, 6 and 7 weeks following ligature placement. •Block biopsies from implant and tooth sites. •Histologic analysis.	•PE was thinner at implant than at tooth sites, terminated at or at varying distances above alveolar bone in PiMs, compared with tooth sites, where no or minimal migration of PE was observed. •ICT size larger and with higher density of lymphocytes at PiM than at tooth sites. •Many osteoclasts and Howship's lacunae in PiM and ankylosed teeth.
Nociti et al. (2001)	•Five dogs. - 20 implants (Napio system). - 20 teeth (maxillary premolars).	•3 months healing after implant placement. •Ligatures for 4 weeks at implants and teeth.	•Clinical examination of implants and teeth on day 0 and 30 days after ligature placement.	•Clinical signs of tissue destruction at both implants and teeth sites with similar rate of attachment loss.
Schou et al. (2002)	•Four cynomolgus monkeys - 8 implants (experimental Astra implants with machined surface). - 8 teeth (second pre-molars or second molars).	•3 months healing after implant placement. •Ligatures secured by orthodontic elastics for 7 months at implants and for 4 months at teeth (replaced or pushed apically once every 4 weeks).	•Block biopsies from implant and tooth sites. •Histologic analysis.	•Apical migration of PE at implant and tooth sites, extensive ulceration only at implant sites. •0.2-0.4mm bone loss, Howship's lacunae and osteoclasts at implant and tooth sites.

PiM: peri-implant mucosa; ICT: inflamed connective tissue; CT: connective tissue; PE: pocket epithelium; PMN: polymorphonuclear cells.

Table 2. Studies comparing human peri-implantitis and periodontitis lesions - clinical, histological analyses

References	Number of subjects/implants/teeth involved	Definition-diagnosis for peri-implantitis/ function time/implant system	Methods	Results
Bullon et al. (2004)	•10 subjects: - 5 subjects with PI (five implants). - 5 subjects with AG (five biopsies from sites with PPD ≥6 mm).	•PPD 4-5 mm, BOP+, radiological evidence of bone loss. •Several months loading (average not specified). •Implant type not specified.	•Soft-tissue biopsies. •Histological and IHC in areas: oral epithelium (O), supracrestal connective tissue (S-J). •IHC (CD1a, CD3, CD20, CD34, factor VIII, VEGF, oncoproteins bcl2 and p53).	•Histological analysis: -multi-layered parakeratinized oral epithelium in PiM and AG sites. -thin nonkeratinized junctional epithelium, partly ulcerated in PIM. •IHC analysis: -O: significantly less CD1a and CD34, but significantly more VEGF and bcl2 in PI than in AG sites. -S–J: significantly more CD34, Factor-VIII and VEGF in PI than in AG sites.
Konttinen et al. (2006)	•20 subjects: -10 subjects with PI (10 implants). -10 subjects with CP (number of gingiva biopsies not specified).	•Pain during mastication and implant mobility and vertical bone loss. •Time in function not specified. •Implant type not specified.	•Soft-tissue biopsies (PiM and gingiva). •IHC (TNF-α, IL-1a, IL-6, PDGF-A, TGF-α).	•Higher percentage of IL-1a and IL-6, lower percentage of TNF-α in PI than in CP sites. •Multinuclear, foreign body giant cells only in PiM (originated from macrophages, produce osteoclast-stimulating cytokines), not in CP sites.
Venza et al. (2010)	•135 subjects: -53 subjects with PI (15 systemically healthy, 18 with type 2 Diabetes Mellitus and good glycemic control, 20 with poor glycemic control and diabetic retinopathy). -82 subjects with CP (25 systemically healthy, 27 with type 2 Diabetes Mellitus and good glycemic control, 30 with poor glycemic control and diabetic retinopathy).	•Moderate PI: PPD 3-4 mm, BOP+, radiological evidence of bone loss involving 4 threads. •Advanced PI: PPD ≥ 5 mm, BOP+, radiological evidence of bone loss involving > 4 threads. •At least 24 months loading (average not specified). •Machined implant.	•Soft-tissue biopsies (PiM and gingiva). •Real-time PCR (TNF-α, IL-6, IL-8, MCP-I, CCR1, CCR2, CCR3, CCR4, CCR5, CXCR1, CXCR2, CXCR3) •Western blot.	•Higher percentage of TNF-α, IL-8, CCR5 and CXCR3 in PI than in CP sites. •Poor glycemic control abolished the difference between CP and PI regarding the expression of these mediators.
Becker et al. (2012)	•14 subjects: -7 subjects with PI (7 implants). -7 subjects with CP (7 biopsies from sites with mild CAL (1-2mm CAL), moderate (3-4mm CAL) or severe (≥ 5mm CAL).	•PPD ≥ 5 mm, BOP+, radiographic bone loss exceeding 3 mm. •At lest 1 year in function. •Implant type not specified.	•Transcriptome analysis.	•PI and CP exhibit significantly different mRNA signatures.

PI, peri-implantitis; AG, aggressive periodontitis; CP, chronic periodontitis; PPD, probing pocket depth; BoP, bleeding-on-probing; IHC, immuno-histochemical; PiM, peri-implant mucosa; CAL, clinical attachment loss

Treatment of peri-implantitis

The primary goals of treatment of peri-implantitis are to resolve inflammation and to arrest the progression of disease. As the aetiology of peri-implantitis is similar to that of periodontitis, anti-infective protocols comparable to those used in the treatment of periodontitis should be adopted to treat peri-implantitis (Lindhe & Meyle, 2008). Thus, decontamination of the implant surface is considered as a priority for the treatment of peri-implantitis. Treatment protocols have often included surgical access to implants presenting with peri-implantitis and numerous protocols including different chemical detergents, air-powder abrasive devices or lasers, have been presented to achieve decontamination of implant surfaces. (Claffey et al., 2008)

Pre-clinical *in vivo* studies in animals

Pre-clinical *in vivo* studies on treatment of experimentally induced peri-implantitis have demonstrated that resolution of peri-implantitis lesions is possible. Animal models of experimental peri-implantitis have been useful for evaluation of various implant surface decontamination protocols in the surgical treatment of peri-implantitis (Table 3). Numerous implant surface decontamination methods as part of the surgical treatment of peri-implantitis have been suggested, either alone or in different combinations, but no single decontamination procedure was found to be superior. Schou et al. (2003) compared 4 methods in a monkey model: (1) air-powder abrasive technique followed by citric acid application, (2) air-powder abrasive technique alone, (3) gauze soaked in saline followed by citric acid application, and (4) gauze soaked alternately in a 0.1 % solution of chlorhexidine digluconate and saline. Experimental peri-implant defects, created over a period of 9 to 17 months around implants with a TPS surface, were surgically exposed. Each implant surface was subjected to one of the previously mentioned treatment procedures. All defects were filled with autogenous bone graft particles and covered by an e-PTFE membrane. Clinical parameters, radiological assessments, histological, and stereological analyses did not reveal significant differences between any of the methods used. It was concluded that for implants with a modified surface, the simplest method, i.e., gauze soaked alternately in chlorhexidine and saline, should be the preferred implant surface decontamination method when combined with membrane-covered autogenous bone graft particles.

Other pre-clinical *in vivo* studies confirmed that resolution of peri-implantitis lesions is possible at implants with modified surfaces by decontamination with gauze soaked in saline (Persson et al., 1999; Persson et al., 2001; Albouy et al., 2011). Albouy et al. (2011), in an experimental study in dogs, reported on the outcome of treatment of peri-implantitis using gauze soaked in saline in the absence of systemic antibiotics. It was concluded that resolution of peri-implantitis following treatment without systemic antibiotics or local antiseptic was possible. However, it was also demonstrated that implant surface

characteristics influenced treatment outcomes with a poorer results at implants with a porous anodized surface (TiUnite) when compared to implants with turned, TiOblast and SLA surfaces.

In **study III**, using a pre-clinical *in vivo* dog model, appropriate radiological, histological and microbiological methods were applied to evaluate resolution of peri-implantitis following surgical treatment at implants with different surface characteristics.

Clinical studies

Prospective studies evaluating outcomes of surgical therapy of peri-implantitis with a follow-up period of at least 1 year, and aiming at comparing different methods of implant-surface decontamination are few. (Table 4)

Although several surgical protocols for treating peri-implantitis have been applied in many case series, there are few randomized controlled trials using a define control treatment. Most studies focused on outcomes of reconstructive procedures comparing different types of reconstructive techniques, different grafting materials and the use of membranes (Schwarz et al., 2006, 2008, 2009; Deppe et al., 2007; Roos Jansåker et al., 2007, 2011, 2014; Romanos & Nentwig, 2009; Aghazadeh et al., 2012). Khoshkam et al. (2013), in a review, concluded that there was currently no evidence of additional benefit of reconstructive procedures over other treatment modalities for managing peri-implantitis. Only few studies have investigated the effect of access flap surgery combined with debridement and implant surface decontamination (Leonhardt et al., 2003; de Mendonça et al., 2009; Duarte et al., 2009; Máximo et al., 2009; Heitz-Mayfield et al., 2012) or resective surgical procedures (Romeo et al., 2005, 2007; Serino & Turri, 2011; de Waal et al., 2013). Regardless of technique, the majority of surgical protocols included administration of perioperative or postoperative systemic antibiotics (Behneke et al., 2000; Leonhardt et al., 2003; Romeo et al., 2005; 2007; Roos Jansåker et al., 2007; 2011; 2014; Roccuzzo et al., 2011; Serino & Turri, 2011; Aghazadeh et al., 2012; Heitz-Mayfield et al., 2012; Wiltfang et al., 2012). However, as concluded in a consensus report from the 8th European Workshop on Periodontology, (Sanz & Chapple, 2012), the influence of the adjunctive use of systemic antibiotics on treatment outcome is still unknown. Thus, adequately powered randomized controlled trials are of high priority (Berglundh & Giannobile, 2013).

In **study IV**, a randomized controlled clinical trial, the effect of the local use of chlorhexidine for implant surface decontamination in surgical treatment of peri-implantitis was investigated and the outcome of surgical therapy of peri-implantitis with and without systemic antibiotics evaluated.

Table 3. Pre-clinical in-vivo studies comparing various implant surface decontamination methods during peri-implantitis surgical treatment.

References	Number of treated subjects/implants	Implant / Surface	Implant surface decontamination	Materials	Systemic antibiotics (drug and duration)	Follow-up	Results
Persson et al. (1999)	- 4 dogs. - 24 implants.	•Brånemark Systems. (Machined).	•Control group : cotton pellets soaked in sterile saline. •Test group : abrasive pumice with rotative brush.	• None.	•Amoxicillin + metronidazole (3 weeks).	•7 months.	•Resolution of peri-implant inflammation and new-bone formation occurred in both decontamination groups. No significant difference was observed between control and test group. •Thin connective-tissue capsule observed between the implant surface and the newly formed bone.
Deppe et al. (2001)	- 6 dogs. - 60 implants.	•Straumann. (TPS).	•Group 1 : Air-powder abrasive. •Group 2 : CO_2 laser. •Group 3 : Air-powder abrasive + CO_2 laser.	•Control group : none (debridement alone). •Test group : ePTFE membrane.	•No.	•4 months.	•No significant differences between groups for bone gain. •Laser groups showed more bone-to-implant apposition than group treated with air-powder abrasive alone.
Kolonidis et al. (2003)	- 4 dogs. - 12 implants.	•Brånemark Systems. (Machined).	•Group 1 : cotton pellets soaked with citric acid (30 sec) + rinsing with saline solution. •Group 2 : toothbrush + saline (1 min). •Group 3 : cotton pellet soaked with 10% hydrogen peroxide (1 min) + rinsing with saline solution.	•None.	•Clindamycine. (1 week).	•11 weeks.	•All treatment modalities were associated with direct bone-to-implant contact on the portion of implant surface previously exposed to the oral environment.
Schou et al. (2003)	- 8 monkeys. - 64 implants.	•Straumann. (TPS).	•Group 1 : Air-powder abrasive + citric acid. •Group 2 : Air-powder abrasive. •Group 3 : gauze soaked with saline + citric acid. •Group 3 : gauze soaked alternately with chlorhexidine and saline.	•Autogenous bone + e-PTFE membrane.	•Ampicillin + metronidazole (12 days).	•6 months.	•Evaluation by clinical parameters, radiography, histology, and stereology did not reveal significant differences between the implant surface decontamination methods.
Persson et al. (2004)	- 4 dogs. - 24 implants.	•Straumann. (Machined / SLA).	•Control group : curettes + cotton pellets soaked in sterile saline. •Test group : curettes + CO_2 laser + hydrogen peroxide solution irrigation.	• None.	•Amoxicillin + metronidazole (17 days).	•6 months	•The amount of re-osseointegration was 21% and 82% at laser-treated machined implants and SLA implants, respectively, and 22% and 84% at saline-treated machined implants and SLA implants, respectively. •The use of CO_2 laser and hydrogen peroxide during surgical therapy had no apparent effect on bone formation and re-osseointegration.

TPS, Titanium Plasma Sprayed surface; CO_2 laser, Carbon dioxide laser; e-PTFE, expanded polytetrafluoroethylene; SLA, Sandblasted Large Acid-etched surface; Er-YAG laser, Erbium-doped yttrium-aluminium-garnet laser.

(Continued).

References	Number of treated subjects/implants	Implant / Surface	Implant surface decontamination	Materials	Systemic antibiotics (drug and duration)	Follow-up	Results
Schwarz et al. (2006)	- 5 dogs. - 30 implants.	•Straumann. (SLA).	1-Er:YAG laser. 2-an ultrasonic device (Vector). 3-plastic curettes + local application of metronidazole gel.	• None.	•No.	•3 months.	•All treatment procedures resulted in statistically significant improvements of all clinical parameters. Radiological improvements were merely observed at implants. treated by open flap debridement. Er:YAG laser seemed to be more suitable to promote re-osseointegration than Vector or plastic curettes + local application of metronidazole gel.
Shibli et al. (2006)	- 5 dogs. - 40 implants	•Implamed. (Machined / TPS). •Biomet 3i. (Osseotite). •Conexão Implants. (microrough).	•Control group : plastic curettes. •Test group : plastic curettes + lethal photosensitiza-tion.	e-PTFE membrane.	•No.	•5 months.	•Re-osseointegration ranged between 31% and 42% but no differences were observed between the different implant surface characteristics.
Takasaki et al. (2007)	- 4 dogs. - 12 implants.	•Straumann. (SLA).	•Control group (6 implants): plastic curettes + sterile saline solution irrigation. •Test group (6 implants): Er-YAG laser + sterile saline solution irrigation.	• None.	•Yes (name of the sys-temic antibiotics not specified) (3 days).	•24 weeks.	•Based on the histological results, both treatments showed significant new bone formation on the treated implant surface. No significant difference was observed between control and test group.
Alhag et al. (2008)	- 4 dogs. - 12 implants.	•Nobel Biocare. (TiUnite).	•Group 1 : cotton pellets soaked with citric acid (30 sec) + rinsing with saline solution. •Group 2 : toothbrush + saline (1 min). •Group 3 : cotton pellet soaked with 10%. hydrogen peroxide (1 min) + rinsing with saline solution.	•None.	•Clindamycine (1 week).	•11 weeks.	•All treatment modalities were associated with direct bone-to-implant contact on the portion of implant surface previously exposed to the oral environment.

TPS, Titanium Plasma Sprayed surface; CO_2 laser, Carbon dioxide laser; e-PTFE, expanded polytetrafluoroethylene; SLA, Sandblasted Large Acid-etched surface; Er-YAG laser, Erbium-doped yttrium-aluminium-garnet laser.

Table 4. Clinical studies comparing various implant surface decontamination methods during peri-implantitis surgical treatment.

References	Number of treated subjects/implants	Implant / Surface	Implant surface decontamination	Materials	Systemic antibiotics (drug and duration)	Follow-up	Results
Romeo et al. (2005, 2007)	- 17 subjects. - 35 implants.	•Straumann. (TPS).	•Control group (7 patients/16 implants): plastic curettes metronidazole gel 25% + tetracycline hydrochloride solution + rubbed 3 min + sterile saline solution. •Test group (10 patients/19 implants): plastic curettes metronidazole gel 25% + tetracycline hydrochloride solution + rubbed 3 min + sterile saline solution + implantoplasty.	• None.	•Amoxicillin. (8 days)	•1, 2, 3 years	•The resective therapy associated with implantoplasty seemed to influence positively the survival and the clinical parameters of implants affected by peri-implantitis. The results were confirmed by the radiological analysis. 3 years after surgical treatment, the cumulative survival rate was 87.5% for the implants of control group and 100% for the implants of test group.
Deppe et al. (2007)	- 32 subjects. - 73 implants.	•Brånemark Systems. •IMZ. •Straumann. •Frialit.	•Group 1 (6 patients/19 implants): Air-powder abrasive alone. •Group 2 (7 patients/15 implants): Air-powder abrasive alone. • Group 3 (10 patients/22 implants): Air-powder abrasive + CO_2 laser. • Group 4 (9 patients/17 implants): Air-powder abrasive + CO_2 laser.	•Group 1 (6 patients/19 implants): none. •Group 2 (7 patients/15 implants): beta-tricalcium phosphate + autogenous bone + e-PTFE membrane. • Group 3 (10 patients/22 implants): Air-powder abrasive + CO_2 laser. • Group 4 (9 patients/17 implants): beta-tricalcium phosphate + autogenous bone + e-PTFE membrane.	•No.	•5 years.	•The treatment of peri-implantitis seemed to be accelerated by using a CO_2 laser concomitant with resective surgery. However, with respect to long-term results in sites treated with beta-tricalcium phosphate + autogenous bone + e-PTFE membrane, no difference was observed between laser and conventional implant surface decontamination.
Schwarz et al. (2011, 2012, 2013)	- 32 subjects. - 35 implants.	•10 different implant systems.	•Bucally and supracrestally exposed implant parts: Implantoplasty. •Remaining intra-bony aspect of the implant surface: 1-Er:YAG laser (16 patients/19 implants). 2-plastic curettes + cotton pellets soaked in sterile saline (16 patients/16 implants).	•Xenogenic bone mineral in combination with a collagen membrane.	•No.	•1, 2 and 4 years.	• Er:YAG-treated sites failed to reveal higher reductions in mean BoP and clinical attachment level values when compared with the group treated with curettes. •Both groups exhibited a comparable radiographic bone fill at the intra-bony defect component. •The long-term stability of clinical outcomes obtained following combined surgical therapy of advanced peri-implantitis may be influenced by factors other than the method of surface debridement/decontamination.

TPS, Titanium Plasma Sprayed surface; CO_2 laser, Carbon dioxide laser; e-PTFE, expanded polytetrafluoroethylene; Er-YAG laser, Erbium-doped yttrium-aluminium-garnet laser; BoP, Bleeding on Probing; SLA, Sandblasted Large Acid-etched surface.

(Continued)

References	Number of treated subjects/implants	Implant / Surface	Implant surface decontamination	Materials	Systemic antibiotics (drug and duration)	Follow-up	Results
De Waal et al. (2013)	- 30 subjects. - 79 implants.	•Astra Tech Imp System (Osseospeed). •Brånemark Systems, (machined). •Straumann. (TPS / SLA/ SLAactive). •IMZ (TPS). •Nobel Biocare (TiUnite). •Friadent (microrough).	•Control group: placebo solution. •Test group: 0.12 chlorhexidine + 0.05% cetylpyridium chloride.	• None.	•No.	•1 year.	•Greater immediate suppression of anaerobic bacteria on the implant surface was reported at implant in test groups, but no superior clinical results was observed.

TPS, Titanium Plasma Sprayed surface; SLA, Sandblasted Large Acid-etched surface.

Aims

The current series of studies has a translational profile and aims at characterizing peri-implantitis lesions and improving methods in treatment of the disease.

The specific aims were:

- to analyze the tissue reactions following ligature removal in experimental periodontitis and peri-implantitis in dogs. (**Study I**)

- to examine differences in cellular characteristics of human peri-implantitis and periodontitis lesions. (**Study II**)

- to evaluate the effect of surgical treatment of experimental peri-implantitis at implants with different surfaces characteristics using different anti-infective procedures. (**Study III**)

- to investigate the adjunctive effect of systemic antibiotics and local use of chlorhexidine for implant decontamination on surgical treatment of peri-implantitis. (**Study IV**)

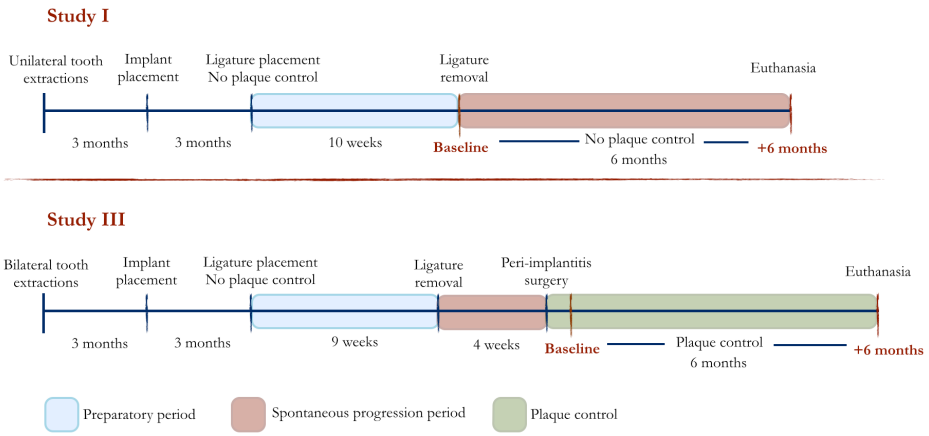
Material & methods

Animal studies (Study I and III) – Study protocol

The protocol of each experiment was approved by the regional Ethics Committee for Animal Research, Göteborg, Sweden (approval Dnr 181-2006 and Dnr 221-2009, respectively). The experiments were conducted at the Laboratory of Experimental BioMedicine at the Sahlgrenska Academy, University of Gothenburg in 2007 and 2011 respectively.

Two groups of 6 destination-bred Labrador dogs about 1,5 year old were used. The animals were fed a soft diet during the experiment. The outline of study I and III are depicted in Figure 1.

Figure 1. Schematic view of the outline of the pre-clinical in vivo studies.



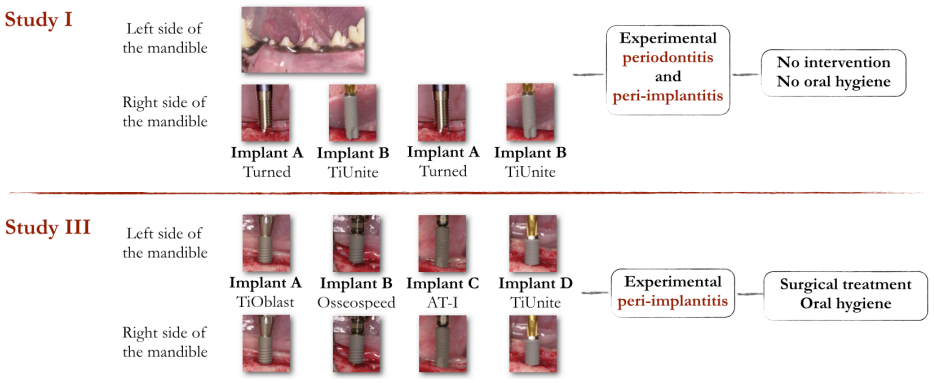
General anesthesia

During all surgical procedures general anesthesia was induced with intravenously injected Propofol (10mg/ml, 0.6ml/kg) and sustained with N_2O:O_2 (1:1.5-2) and Isoflurane employing endo-tracheal intubation.

Implant placement

The mandibular premolars and the first molar and the three anterior premolars of the maxilla were extracted in all dogs on the right side in study I and bilaterally in study III. Three months later, 4 implants were placed in a randomized order in the edentulous premolar area of the mandible. (Figure 2)

Figure 2. Design of the pre-clinical in vivo studies.



In study I, 4 implants with similar geometry and with two different surface characteristics (MKIII NP, 3.3 x 10 mm, Nobel Biocare AB, Göteborg, Sweden / implant A; turned surface and implant B; TiUnite™ surface) were placed pair-wise in the right side of the mandible. One dog developed Adisson's disease and was euthanized 2 months after implant installation.

In study III, 4 implants with different surface characteristics were used: implants A, B and C had the dimension 3.5 x 11mm (ASTRA TECH Implant SystemTM, Dentsply Implant, Mölndal, Sweden) and presented respectively a TiOblast™ surface (implant A), an Osseospeed™ surface (implant B) and a AT-I surface (Johansson et al., 2012) (implant C). Implant D had the dimension 3.3 x 11.5mm with a TiUnite™ surface (NobelBiocare AB, Göteborg, Sweden). The sequence of implant placement was identical in both sides of each animal but randomized between animals.

Experimental periodontitis and peri-implantitis

Three months after implant installation, experimental peri-implantitis was initiated around all implants in both experimental studies. In study I, experimental periodontitis was also initiated around the 4th, 3rd and 2nd premolars in the left side of the mandible. Plaque control procedures were abandoned and cotton ligatures were placed in a sub-gingival position around teeth and in a corresponding position around the neck portion of the implants in a manner previously described (Lindhe et al. 1992, Zitzmann 2004).
The ligatures were removed and a new set of ligatures was placed in a more apical position at all sites after 3 weeks. The ligature shift procedure was repeated 3 weeks later and the ligatures were finally removed at 9 weeks (study III) and 10 weeks (study I) after the initiation of the experimental breakdown.

Spontaneous progression of experimental periodontitis and peri-implantitis (Study I)

After ligature removal, plaque accumulation was allowed during a subsequent 26-week period.

Surgical treatment of experimental peri-implantitis (Study III)

Oral hygiene procedures were re-instituted at all implants immediately after ligature removal. Treatment of peri-implantitis was performed at all implants four weeks later. No systemic antibiotics were administrated. The treatment included open flap debridement/ decontamination of the implant. Two different implant surface decontamination procedures, saline (control group) or a 0.2 % solution of chlorhexidine digluconate (test group), one on each side of the mandible, were randomly and equally allocated in a split-mouth design. Thus, full-thickness flaps were raised on the buccal and lingual aspects of all implants and the inflamed tissue within the crater-formed bone defects was removed. If present, calculus was removed from the implant surface by the use of curettes. In one side of the mandible, the implants were carefully cleaned for 3 minutes by sterile 10 x 10 mm gauze soaked in saline, while in the contralateral side cleaning of implants was performed using sterile 10 x 10 mm gauze soaked in a 0.2% solution of chlorhexidine digluconate. The flaps were repositioned and sutured. The sutures were removed after 2 weeks and mechanical infection control procedures were re-instituted and maintained during the subsequent 6-month period of the experiment.

Radiological and clinical examination

For all animals, radiological and clinical examinations of tooth and implant sites were performed during the active breakdown period and at ligature removal. A set of radiographs was obtained from tooth and implant sites using a customized film holder (Kerr Hawe, Bioggio, Switzerland) as previously described by Persson et al. (1999) and Albouy et al. (2009, 2011).

In study I, radiographs were obtained 10, 16 and 26 weeks after ligature removal (baseline). In study III, clinical and radiological examinations were performed and repeated at 2 weeks (baseline) and 2, 3, 4 and 6 months after surgery.

Microbiological sampling (Study III)

In study III, microbiological samples were obtained from all experimental peri-implantitis sites 4 weeks after ligature removal and at 3 and 5 months of follow-up.

Cotton rolls were used to isolate the experimental areas to avoid saliva contamination. Supra-gingival plaque was removed by a sterile gauze soaked in saline. Four sterile medium sized paper points (Dentsply, Maillefer, size 35, Ballaigues, Switzerland) were inserted into the most apical part of the peri-implant pocket and held in place for 10 seconds. The paper points were removed and placed in Eppendorf tubes (Starlab, Ahrensburg, Germany)

and prepared for microbiological analysis (checkerboard DNA-DNA hybridization technique).

Biopsy procedure

26 weeks after ligature removal (study I) or after peri-implantitis surgery (study III), the dogs were euthanized with a lethal dose of Sodium-Pentothal® (Hospira Enterprises B. V., Hoofddorp, Netherlands) and perfused through the carotid arteries with a fixative (4 % formaldehyde). The mandibles were retrieved, and tissue blocks from tooth- and implant sites were dissected using a diamond saw (Exakt, Kulzer, Norderstedt, Germany) and stored in the fixative.

In study I, two blocks were produced from the tooth site of the mandible: one posterior block containing the 4th premolar and the distal root portion of the 3rd premolar and one anterior block containing the 2nd premolar and the mesial root portion of the 3rd premolar. Using a randomization protocol, 50 % of the tissue blocks from tooth and implant sites were processed for ground sectioning according to the methods described by Donath & Breuner (1982) while the remaining samples were decalcified and embedded in paraffin (tooth sites) or further prepared according to the "fracture-technique" (implant sites) (Berglundh et al., 2004) and embedded in paraffin.

In study III, all tissue specimens were processed for ground sectioning.

Human biopsy samples and clinical study (Study II and IV) - Study protocol

The protocols of study II and IV were approved by the regional Ethics Committee, Göteborg, Sweden (approval Dnr 245-10 and Dnr. 654-10, respectively). All subjects were informed about the study, given a detailed description of the procedure and signed a written consent.

Power calculation

In study II, for superiority of peri-implantitis lesions in relation to periodontitis lesions, with an α of 0.05, a given standard deviation of 1.8 %, and a power of 80 %, a difference in area proportions of cells of 3 % required a sample size of 30 subjects in each group. To compensate for possible complications during histological processing, the number of recruited patients was 40 for each group.
In study IV, sample size calculation was based on a difference of PPD reduction between groups of 0.5 mm with a standard deviation (S.D.) of 0.5 mm, a significance level of 5 % and 80 % power. The required sample size was 20 subjects for each treatment group.

Study II

Two groups of patients from one clinic in periodontics, Mölndal, Public Dental Health Services, Region Västra Götaland, Sweden, were included. One group consisted of 40 patients with generalized severe chronic periodontitis (24 women and 16 men; age range, 40-89 year; mean, 64 ± 11.45 year). The patients exhibited bone loss ≥ 50 % and probing pocket depth ≥ 7 mm with bleeding on probing at ≥ 4 teeth. A second group of 40 patients presenting with severe peri-implantitis was also recruited (23 women and 16 men; age range, 46-93 year; mean, 70 ± 10.41 year; function time for implants, 2-10 year). The subjects in this group demonstrated at least 1 implant with peri-implant bone loss ≥ 3 mm and a peri-implant probing pocket depth ≥ 7 mm, with bleeding on probing and/or suppuration.

None of the subjects had a known systemic disorder that could have affected the periodontal and peri-implant tissue conditions. Smoking habits were recorded in both groups. No patients had received any treatment regarding periodontal or peri-implant diseases during the last 6 months.

Biopsy procedures

Diseased interproximal tooth/implant sites were identified that exhibited probing pocket depth ≥ 7 mm with bleeding on probing. Following local anesthesia (Xylocain Dental Adrenalin, 20 mg/mL + 12.5 μg/mL; Dentsply Pharmaceutical, York, PA, USA), 2 parallel incisions, 4 mm apart, were made with a 12D scalpel blade (Hu-Friedy®, Chicago, IL, USA) through the soft tissue until bone contact was achieved. The 2 incisions were connected with a perpendicular incision placed at a distance of 4 mm from the tooth/implant. The biopsies, including the entire supracrestal soft tissue portion of the diseased site, were carefully retrieved, mounted in mesh basquets (Tissue-Tek® Paraform® Sectionable Cassette System, Inc. Sakura Finetek Europe, The Netherlands) and placed in 4 % buffered formalin for 48h. The samples were stored in 70 % ethanol and kept at 4°C.

Study IV

The study was registered at *ClinicalTrials.gov* (NCT01857804). CONSORT guidelines for clinical trials were followed and the study flow chart is presented in Figure 3.

The study population consisted of 100 patients (35 males and 65 females; mean age 66.3 ± 13.6 years) presenting with severe peri-implantitis at one or more implants (i.e. peri-implant probing pocket depth ≥6 mm on at least one aspect of the implant, together with bleeding and/or suppuration on probing (BoP and/or SoP positive) and radiographically documented marginal bone loss of >3 mm).

The patients were referred to two specialist clinics in periodontics (Mölndal and Gothenburg, Public Dental Health Services, Region Västra Götaland, Sweden) and were enrolled between October 2010 and December 2013.

Exclusion criteria were compromised general health, treatment with systemic antibiotics during the past 6 months and a known allergy to penicillin.

Baseline examination and randomization procedure

In the baseline examination, the following variables were recorded at the mesial, distal, buccal and lingual aspects of each implant: probing pocket depth (PPD) measured with a manual periodontal probe (Hu-Friedy®, Chicago, IL, USA), BoP/SoP within 15 seconds following pocket probing.

Patients were randomly allocated to four treatment groups using computer-generated lists: *Group 1* (systemic antibiotics/implant surface decontamination with antiseptic agent) (n=27), *Group 2* (systemic antibiotics/implant surface decontamination with saline) (n=25), *Group 3* (no systemic antibiotics/implant surface decontamination with antiseptic agent) (n=24) and *Group 4* (no systemic antibiotics/implant surface decontamination with saline) (n=24).

The allocation procedure was stratified for smokers/non-smokers. Demographic data of the patient sample are presented in Table 5. The distribution of implant-categories with regard to surface characteristics between treatment groups is depicted in Table 6. 24 % of all implants had a non-modified surface (category A). In patient groups 1 and 2, the 10-day systemic antibiotic regimen (amoxicillin 2 x 750mg daily) commenced 3 days prior to surgery. In patient groups 1 and 3 an antiseptic agent (0.2 % solution of chlorhexidine digluconate) was applied for implant surface decontamination during surgery.

Microbiological sampling and analysis

Samples from the subgingival microbiota were obtained at implant sites targeted for surgical therapy. The area of the sites chosen for sampling was isolated with cotton rolls, dried and supra-gingival plaque was removed with sterile cotton pellets. 6 sterile paper points (Dentsply, Maillefer, size 35, Ballaigues, Switzerland) were inserted to the most apical part of the peri-implant pocket, kept in place for 10s and then placed in two different tubes for culture and checkerboard DNA–DNA hybridization analysis, respectively.

Surgical procedure

Prior to surgery, patients were enrolled in a hygiene program including professional supragingival implant/tooth cleaning using rubber cups, polishing paste and oral hygiene instructions. The surgical procedure was aiming at pocket elimination using resective techniques. Screw-retained supra-constructions were removed. Following local anesthesia, full thickness flaps were elevated on the buccal and lingual aspects of affected implants. Inflamed tissue was removed and titanium-coated curettes (Hu-Friedy®, Chicago, IL, USA) were used to remove hard deposits on implants.

Figure 3. CONSORT flow chart of the study.

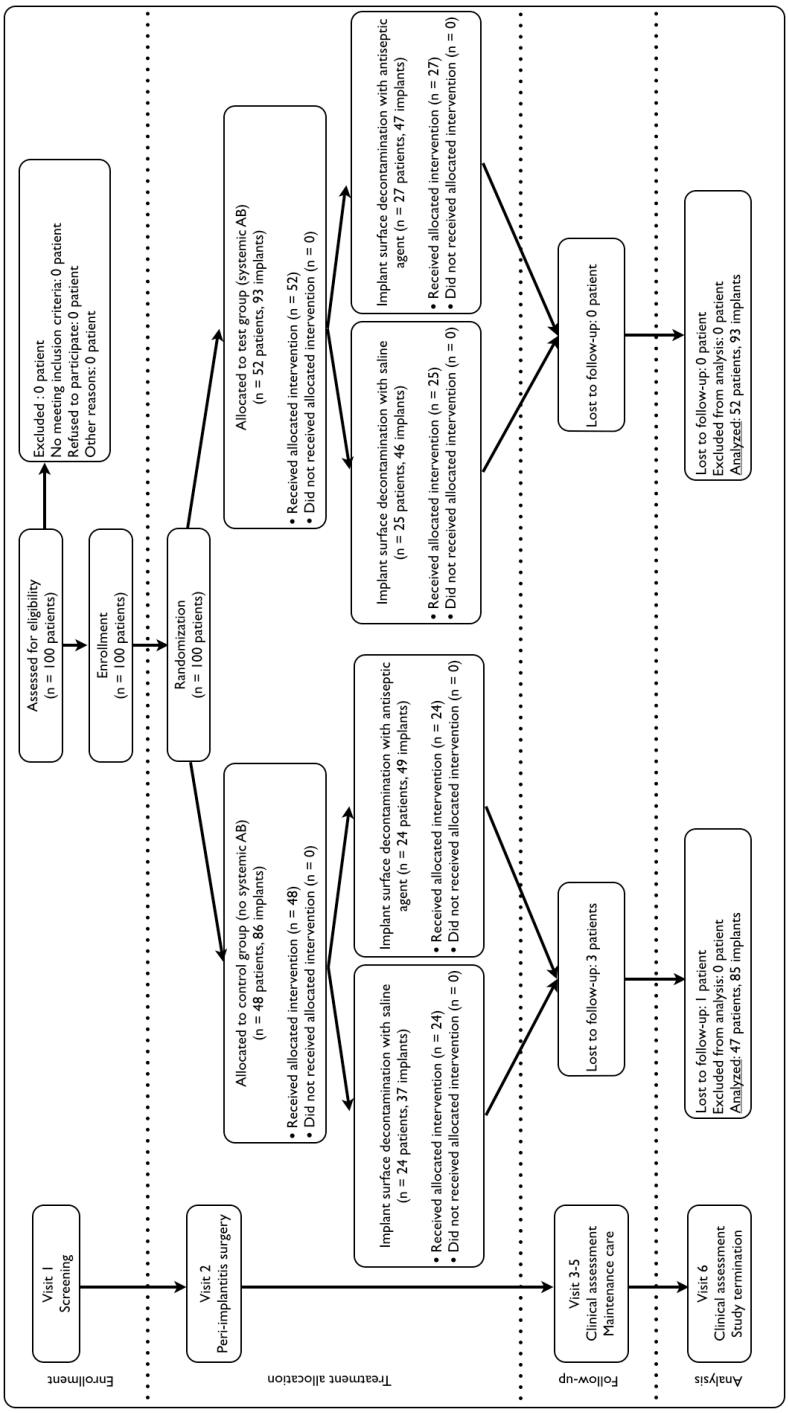


Table 5. Demographic data on patients.

		All groups	Group 1 (AB+/AS+)	Group 2 (AB+/AS-)	Group 3 (AB-/AS+)	Group 4 (AB-/AS-)
Number of patients		100	27	25	24	24
Age years; mean (range)		66.3 (21-90)	65.7 (23-90)	67.9 (21-88)	64.6 (27-81)	66.9 (30-88)
Gender n (%)	Male	35	7 (25.9)	8 (32)	10 (41.7)	10 (41.7)
	Female	65	20 (74.1)	17 (68)	14 (58.3)	14 (58.3)
Smoking habits n (%)	Smoker	33	9 (33.3)	9 (36)	8 (33.3)	7 (29.2)
	Non-smoker	67	18 (66.7)	16 (64)	16 (66.7)	17 (70.8)
History of periodontitis n (%)		84	21 (77.8)	21 (84)	21 (87.5)	21 (87.5)
Diabetes n (%)		5	2 (7.4)	0	1 (4.2)	2 (8.3)
CVD-related drug therapy n (%)		31	9 (33.3)	8 (32)	6 (25)	8 (33.3)

CVD: Cardiovascular disease

Table 6. Characteristics of affected implants.

			All groups	Group 1 (AB+/AS+)	Group 2 (AB+/AS-)	Group 3 (AB-/AS+)	Group 4 (AB-/AS-)
Number of implants presenting with peri-implantitis (range)			179 (1-7)	47 (1-5)	46 (1-6)	49 (1-7)	37 (1-6)
Jaw n (%)	Maxilla		116 (64.8)	35 (74.5)	28 (60.9)	32 (65.3)	21 (56.8)
	Mandible		63 (35.2)	12 (25.5)	18 (39.1)	17 (34.7)	16 (43.2)
Location n (%)	Anterior (incisor-canine)		91 (50.8)	25 (53.2)	23 (50)	26 (53.1)	17 (45.9)
	Posterior (premolar- molar)		88 (49.2)	22 (46.8)	23 (50)	23 (46.9)	20 (54.1)
Implant surface category n (%)	Non-modified	A	43 (24)	3 (6.4)	12 (26.1)	15 (30.6)	13 (35.1)
	Modified	All modified	136 (76)	44 (93.6)	34 (73.9)	34 (69.4)	24 (64.9)
		B	87	30	21	26	10
		C	9	2	2	1	4
		D	24	7	6	4	7
		E	13	5	5	1	2
		F	3	0	0	2	1

A : Turned surface (Nobel Biocare AB, Göteborg, Sweden); B : TiUnite surface (Nobel Biocare AB, Göteborg, Sweden); C : TiOblast surface (Astra Tech Implant System™, Dentsply Implant IH AB, Mölndal, Sweden); D : Osseospeed surface (Astra Tech Implant System™, Dentsply Implant IH AB, Mölndal, Sweden); E : SLA surface (Straumann, Institute Straumann, Basel, Switzerland), F : Neoss ProActive surface (Neoss Ltd., Harrogate, UK).

Implant surfaces were decontaminated with 10 x 10 mm gauze soaked in either a 0.2 % solution of chlorhexidine digluconate (groups 1 and 3) or saline (groups 2 and 4) for 2 minutes. Osseous recontouring was performed when indicated. The flaps were closed with interrupted sutures and supra-constructions were reconnected. Patients rinsed for 1 minute with 0.2 % chlorhexidine solution twice daily for 14 days following surgery. Sutures were removed two weeks after surgical therapy and self-performed mechanical infection control procedures were initiated. Intra-oral radiographs were obtained using the long-cone paralleling technique and a Dürr Dental digital radiography sensor (Dürr Dental AG, 74321 Bietigheim-Bissingen, Germany) with sensor holder (Eggen-holder or Super-bite blocks, Kerr Dental / Kerr Corporation, Orange, CA, USA).

Evaluation at 6 and 12 months following treatment

During the 12-month follow-up period supra-gingival polishing was performed and oral hygiene reinforced, if indicated, in a 3-month interval. Microbiological samples were taken at 3, 6 and 12 months after surgery. At 6 and 12 months, clinical assessments of PPD, BoP and SoP were performed. In addition, new intra-oral radiographs were obtained at the 12-month examination. Adverse events throughout the study period were also recorded.

Radiological analysis

Study I and III

The radiographs were analyzed in an Olympus SZH10 stereo macroscope (Olympus optical co, GmbH, Hamburg, Germany) and digital images were obtained with a Leica DFC280 camera (Leica, GmbH, Wetzlar, Germany). Calibration of the measurements was performed using a millimeter ruler. The abutment-implant junction at implant sites and the cemento-enamel junction at tooth sites were used as reference landmarks for the radiographic measurements. The vertical distance between the reference landmark and the marginal bone level was assessed at the mesial and distal aspects of each implant/tooth using the QWin software (Leica Qwin Standard V3.2.0, Leica Imaging Systems Ltd., Cambridge, U.K.).

Study IV

The radiographs were analyzed with an image-software (ImageJ64, National Institutes of Health, Bethesda, MD, USA). The known inter-thread pitch distance of the implant was used in each radiograph for the calibration of the coronal-apical measurements. The marginal bone level was assessed at the mesial and distal aspects of each implant at x 10 magnification on a high definition monitor. All radiologic assessments were performed by one investigator (OC).

Histological processing and analysis

Ground sectioning (Study I and III)

The tissue blocks selected for ground sectioning were dehydrated in increasing grades of ethanol and embedded in Technovit 7200 VLC-resin (Kulzer, Friedrichsdorf, Germany) and prepared as described previously (Albouy et al., 2012). From each block (tooth and implant), 2 parallel sections were obtained in a mesio-distal plane and 2 parallel sections obtained in a bucco-lingual plane. The sections were reduced by microgrinding (Exakt, Apparatebau, Norderstedt, Germany) to a final thickness of about 30 μm and stained in toluidine blue and fibrin stain of Ladewig (Donath & Breuner, 1982). All sections were exposed to histometric analysis.

The histological examinations were performed in a Leica DM-RBE microscope (Leica, Heidelberg, Germany) equipped with an image system (Q-500 MC, Leica, Wetzlar, Germany). The following landmarks were identified and used for the linear measurement: the gingival/peri-implant mucosa margin (GM/PM), the abutment–fixture junction (A/F) at implant sites, the cemento-enamel junction (CEJ) at tooth sites, the apical termination of the biofilm (aPlaque) on the implant/tooth surface, the apical termination of the pocket epithelium (aPE), the marginal position of bone closest to the implant/tooth (B), the most coronal extension of the bone crest (BC) and the coronal and apical extension of the infiltrated connective tissue (cICT and aICT).

In study I, the distance between the ICT and the lateral bone wall of the intra-bony defects (ICT-Bw) was measured in three locations; coronal, middle, apical. The surface area of the ICT (ICT area) in the connective tissue was evaluated by outlining its circumference.
In study III, when indicated, areas of the residual intra-bony defect (defined by the bone wall between B and BC) and of an ICT were identified and traced. The occurrence of the ICT was scored as follows:
- *Score 0*: no or only scattered inflammatory cells identified in an area < 1 mm^2
- *Score 1*: scattered inflammatory cells located in an area < 2 mm^2
- *Score 2*: clusters of inflammatory cells presented in infiltrates of a total area < 3 mm^2
- *Score 3*: abundance of inflammatory cells in a total ICT area >3 mm^2

Paraffin-embedded preparation (Study I and II)

Tissue samples that included the implant and the surrounding soft and hard peri-implant tissues (study I), were placed in EDTA and subsequently processed using "the fracture-technique" as described by Berglundh et al. (1994). The specimens were dehydrated and embedded in paraffin (study I and II). Microtome serial sections (5μm thick) were cut and mounted on glass poly-D-lysine-coated slides.

In study I, sections from the implant units were produced parallel with the long axis of the implant, while the tooth units were sectioned in a mesio-distal (P2-P3 or P3-P4) and a bucco-lingual plane (mesial root of P2 or distal root of P4). The paraffin-embedded sections were processed for immunohistochemical preparation.

Immunohistochemistry (Study I and II)

The panel of monoclonal antibodies that were used is presented in Table 7.

Table 7. The panel of antibodies used for the immunohistochemical analysis.

Antibodies	Clone		Specificity	Dilutions	
	Study I	Study II		Study I	Study II
CD3	rabbit	mouse	T-cells	1:200	1:50
CD20	rabbit	mouse	B-cells	1:800	1:400
CD34		mouse	endothelial cells		1:100
CD68		mouse	macrophages		1:200
CD138		mouse	plasma cells		1:50
MPO	rabbit	rabbit	polymorphonuclear leukocytes	1:1000	1:1500
IgG	rabbit		IgG-positive cells (plasma / B cells)	1:100	

In study I, the enzymatic activity of tartrate resistant acid phosphatase (TRAP; acid phosphatase, leukocyte kit, Sigma-Aldrich Inc., St. Louis, MO, USA) was used as a marker for osteoclasts.

The sections were de-waxed and incubated in antigen retrieval solution (DIVA; Biocare Medical, Concord, CA, USA) at 60°C over night and subsequently incubated with primary antibodies for 30 minutes. The specimens were then incubated with a characterized and diluted mouse or rabbit primary antibody, followed by a labeled polymer for 30 minutes and a substrate/chromogen for 10 minutes. Counterstaining was performed with hematoxylin. Finally, the sections were mounted and coverslipped. Human oral mucosa tissue sections were used in Study II as positive controls, while negative controls were produced by substituting the primary antibody with non-immune serum.

The surface area of the infiltrated connective tissue (area ICT) was evaluated by outlining its circumference. The histological quantitative assessments of cell markers were performed using a microscope equipped with an image system (Leitz DM-RBE Q-500 MC® image system, Leica, Wetzlar, Germany). For the identification of positive cell markers, an interference contrast setting at a magnification of x 400 was applied as previously described (Liljenberg et al., 1994; Zitzmann et al., 2001). A point counting procedure was

used to determine the percentage of positive cell markers within the ICT. A lattice comprising 400 points was superimposed over the tissue area. Cross points that indicated the positive cell markers in the compartment to be examined were counted and related to the total counts for the entire ICT (%) and expressed as area proportions (%) of ICT.

In study I, the number of TRAP-positive cells within a 200 μm-wide zone immediately lateral to the bone crest was assessed. The number of TRAP-positive cells/mm in contact with the bone crest was also determined.
In study II, in addition for the point counting procedure, the mean size of positive cells was assessed in 10 randomly selected sections of each category of markers in both patient groups. Based on the data on cell density, size of ICT and cell size, the total number of positive cells for each marker in the ICT was estimated. The density of vascular structures of the ICT was determined using the point counting procedure and the endothelial marker CD34. The density of vascular units was performed in a 200-μm-wide zone of the connective tissue immediately lateral to the ICT.

Microbiological processing and analysis

Checkerboard DNA-DNA hybridization technique (Study III and IV)

Microbial samples scheduled for checkerboard DNA–DNA hybridization were placed in sterile Eppendorf tubes and analyzed according to the checkerboard methodology (Socransky et al., 1994), as modified by Papapanou et al. (1997). They were transferred to 100 μl TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 7.6) and 100 μl 0.5 M NaOH was added and the suspensions boiled for 5 min. After boiling, 800 μl 5 M ammonium acetate was added to each tube and the samples were processed according to standardized procedures. The checkerboard panel included 10 dogs strains (*Pasteurella stomatis, Porphyromonas* sp, *Porphyromonas cangingivalis, Porphyromonas crevioricanis, Porphyromonas gulae, Tannerella forsythia* (dog), *Peptostreptococcus canis, Filifactor villosus, Campylobacter oricanis)* and two human strains (*Prevotella intermedia*, *Treponema denticola*) in study III. In study IV, the panel included 12 human strains (*Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*, *Filofactor alocis*, *Fusobacterium. nucleatum*, *Parvimonas micra*, *Prevotella intermedia/Prevotella nigrescens*, *Prevotella tannerae*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*). The hybrids formed between the bacterial DNA and the probes were detected by application of an antidigoxigenin antibody conjugated with alkaline phosphatase and incubation with a chemiluminiscent substitute (CSPD; Boehringer-Mannheim, Phoenix, AZ, USA).
The obtained chemiluminiscent signals were transformed into a scale of scores from 0 to 5 according to Papapanou et al. (1997): score 0 (no detected signal), score 1 and 2 (signal ≤ 10^5 bacteria) and score 3, 4 and 5 (signal > 10^5 bacteria). The total DNA-probe count was calculated by summing the absolute counts of the separate probes included in the panel.

Culture technique (Study IV)

Microbial samples scheduled for culture were placed in glass bottles containing 3.3 ml VMGA III (Dahlén et al., 1993) and transported to the laboratory for analysis. After mixing a volume of 0.1 ml of the concentrated transport medium to 1:100 and 1:10,000 times dilution in VMGA III, bacteria were plated onto the surface of an enriched Brucella blood agar plate (BBL; Microbiological System, Cockeysville, MD, USA). The agar plates were incubated anaerobically in jars using the hydrogen combustion method (Möller & Möller, 1961) at 37°C for 6–8 days for calculating the total viable count (TVC). *Porphyromonas gingivalis* was distinguished from *Prevotella intermedia/nigrescens* by its haemagglutinating activity and lack of auto-fluorescence in UV light (Slots and Genco, 1979; Slots and Reynolds, 1982). Blood agar (Difco), Staphylococcus agar (Difco), Enterococcus agar (BBL) and tryptic soy serum bacitracin vancomycin agar plates (BBL) were inoculated and incubated for 2 and 5 days, respectively, at 37°C in air with 10 % CO_2. Special attention was given to *Staphylococcus aureus*, *Staphylococcus epidermidis*, enterococci and aerobic Gram-negative bacilli (AGNB). *S.aureus* was distinguished from *S.epidermidis* by performing DNase test on special DNA agar plate (Difco). The plates were examined for typical colony morphology and the specific bacteria were registered as percentage of TVC.

The cut-off score for this semi-quantification were based on a previously published study (Charalampakis et al., 2012) and a 5-graded scale was used to frame the magnitude of bacteria (*Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia/nigrescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, enterococci, AGNB) as proportions of TVC (Dahlén et al 1982): score 0: non-detectable growth of colonies, score 1: <0.1% TVC, score 2: 0.1–1% TVC, score 3 (moderate growth of colonies): 1–10% TVC and score 4: >10% TVC (heavy growth of colonies).

Error of methods

For accuracy assessments of the radiological, histological and immunohistochemical analyses, double measurements were performed in all studies. (Table 8)

Table 8. Inter- and intra-examiner variations.

	Inter-examiner variation mean (S.D.)	Intra-examiner variation mean (S.D.)
Radiological analysis : radiographs (60 % in study I, 40 % in study III, 30 % in study IV) were randomly selected and double assessments performed with a 2-month interval.		
Study I	0.28 mm (±0.24)	0.42 mm (±0.32)
Study III		0.06 mm (±0.11)
Study IV	0.37 mm (±0.49)	0.35 mm (±0.22)

Table 8. Inter- and intra-examiner variations.

		Inter-examiner variation mean (S.D.)	Intra-examiner variation mean (S.D.)
Histological analysis : in randomly chosen sections (25 % in study I and III), one parameter of each assessment category was randomly selected and re-measured.			
Study I	PM/aJE	0.12 mm (±0.13)	0.15 mm (±0.13)
	ICT area	0.75 mm^2 (±0.48)	0.21 mm^2 (±0.19)
Study III	aJE/B		0.18 mm (±0.17)
	ICT area		0.13 mm^2 (±0.27)
Immunohistochemical analysis : in randomly selected sections (45 % in study I, 12 % in study II), the area proportions of cells markers in the ICT were re-assessed. The intra-examiner variations were expressed as mean % (S.D.) on average for cell markers.			
Study I			0.45 % (±0.41)
Study II			0.79 % (±0.56)

Data analysis

The SPSS 21.0 software package (SPSS 21.0 software package, SPSS Inc., Chicago, Illinois, USA) was used for all statistical analysis.

Study I and III

Mean values for all variables were calculated for each implant/tooth unit in each animal. Using the animal as the statistical unit, differences were analyzed using analysis of variance (ANOVA) and the Student–Newman–Keuls test. A p-value <0.05 was considered as significant. A statistical package specially designed for multilevel modeling (MLwiN 2.28; Center for Multilevel Modelling at University of Bristol, Bristol, UK) was used to investigate the influence of dogs, implant/tooth, sites and implant surface-related covariates on the outcome variables.

Study II

Mean values and standard deviations were calculated for each variable and patient. Differences between patient groups were analyzed with the Student's t-test for unpaired observations (n = 80). The null hypothesis was rejected at $p < 0.05$. Analysis of covariance was performed to analyze possible effects of gender, age and smoking on the results.

Study IV

Clinical variables at baseline, 6 and 12 months were expressed in mean values and frequency distributions. Differences were analyzed using analysis of variance, Chi-Square

(between groups) and McNemar analysis (within groups). A p-value <0.05 was considered as significant.

Implant sites presenting with PPD ≤ 5mm, absence of BoP and SoP at the 12 months examination and bone loss ≤ 0.5 mm between 2 weeks and 12 months after surgical therapy, were considered as treatment success and the primary outcome variable. To identify factors affecting the probability of treatment success, a binary logistic regression was used. The independent factors examined included treatment factors, patient-related data (age, gender, smoking habits, history of periodontitis, systemic disorder), implant-related data (number of affected implants, jaw and location). Implants were further categorized according to surface characteristics (non-modified and modified). All variables were tested by the Wald test in a bivariate analysis and statistically significant variables (p<0.05) were retained in the multiple model. The two treatment factors were forced into the final model and possible interaction between factors was explored. Results were expressed as odds ratios (OR) including 95 % confidence intervals.

Results

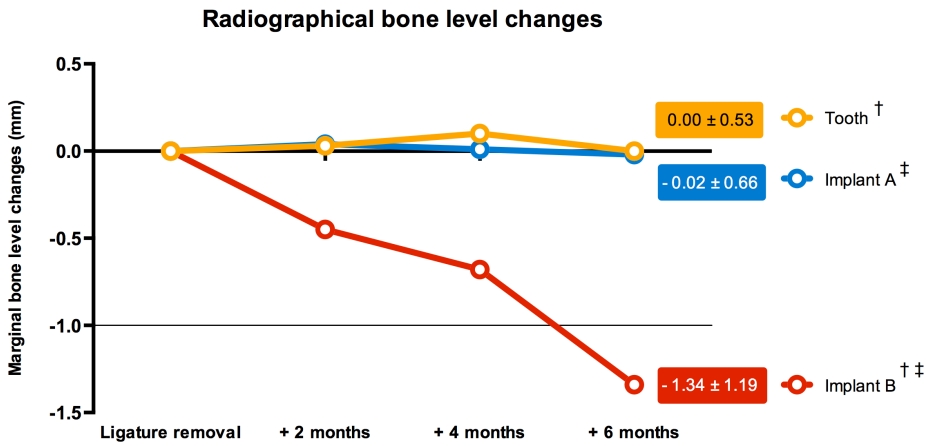
Comparison peri-implantitis/periodontitis (Study I and II)

Radiological findings (Study I)

The mean bone loss that took place during the active breakdown period was significantly greater at both types of implants than at teeth (2.69 ± 0.57 mm for implants in group A, 3.14 ± 0.69 mm for implants in group B and 1.74 ± 0.53 mm for teeth).

The amount of bone loss that occurred during the 26-week period between ligature removal and biopsy is illustrated in Figure 4. The differences between implant B and implant A and between implant B and teeth were statistically significant. Multilevel modeling revealed that neither animal nor implant position in the mandible influenced results.

Figure 4. Radiographical bone level changes after ligature removal.



† p-value<0.05 between tooth and implant B; ‡ p-value<0.05 between implant A and implant B

Histological findings (Study I)

Tissues samples from the experimental model provided access to the entire lesion, including soft and hard tissues.

The examination of the supra-crestal soft tissues portion revealed signs of established disease with greater loss of connective tissue attachment and larger area of ICT in peri-implantitis than in periodontitis lesions. An intact epithelial apical seal and a zone of

structurally intact and non-inflamed connective tissue was consistently present between the apical border of the ICT and the alveolar bone crest in tooth sections. At implant sites, in the contrary, no epithelial barrier was present and the ICT extended to the bone crest.

The examination of the peri-implant tissues revealed an extensive osseous defect, the surface of which was lined with large, multi-nuclear cells. Such cells were only occasionally identified at the alveolar bone surface in the tooth sections.

Results from the histometric measurements at tooth and implant sites are depicted in Figure 5. Overall, vertical dimensions of the pocket epithelium (GM/PM-aPE) and the ICT (cICT-aICT) were significantly larger at implants than at teeth. These dimensions were, in addition, also significantly larger at implants type B than at implants type A. Similar differences were also found with regard to the size of ICT (ICT area), which was significantly closer to the bone (aICT-B) at implants than at teeth. Size and vertical dimension of the intra-bony component was significantly larger at implant B than at implant A.

Immunohistochemical findings (Study I and II)

Common markers for Study I and II

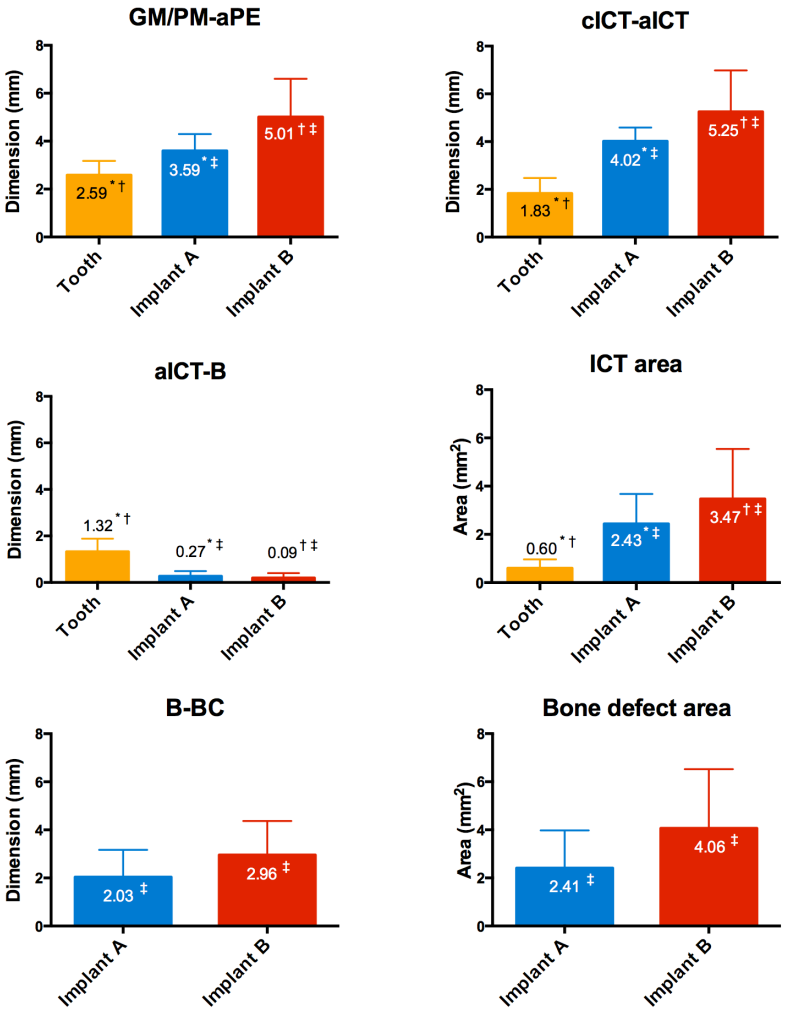
The results from the immunohistochemical analysis are illustrated in Table 9.

Table 9. Size (mm^2) and area proportions of ICT for positive cells in periodontitis and peri-implantitis sites.

	Study I			*Study II*	
	Periodontitis (n=10)	**Peri-implantitis**		**Periodontitis** (n=40)	**Peri-implantitis** (n=40)
		Implant A (n=10)	Implant B (n=10)		
Area (mm^2)					
ICT area	0.42 (±0.28) #,†	1.98 (±1.54) #	2.30 (±0.95) †	1.49 (±1.05)	3.48 (±2.54) *
Cell markers					
CD3 (%)	5.39 (±3.92)	5.78 (±2.11)	7.08 (±3.42)	7.82 (±5.36)	6.87 (±4.42)
CD20 (%)	4.42 (±4.02)	2.61 (±2.82)	1.81 (±1.54)	4.97 (±5.23) *	3.10 (±2.79)
MPO (%)	2.72 (±1.49) #,†	8.53 (±5.71) †,‡	13.26 (±5.81) †,‡	4.28 (±2.52)	10.90 (±7.53) *

p-value<0.05 between tooth and implants A; † *p*-value<0.05 between tooth and implants A;
‡ *p*-value<0.05 between implant A and implants B; * *p*-value<0.05 between human periodontitis and peri-implantitis sites

Figure 5. Results from the histometric measurements at tooth and implant sites. Mean values.



**p-value<0.05 between tooth and implant A; † p-value<0.05 between tooth and implant B; ‡ p-value<0.05 between implant A and implant B.*

In both study I and II, the size of ICT in the peri-implantitis specimens was significantly larger than that of the lesions in the periodontitis sections. The area proportion of the ICT that was occupied by MPO-positive cells was significantly larger in peri-implantitis than in periodontitis specimens in the experimental and the human biopsy study. The density of CD20-positive cells was larger in periodontitis than peri-implantitis lesions in the

human material of study II. No difference were observed between groups regarding CD3-positive cells.

TRAP-positive cells (Study I)

The total number of TRAP-positive cells/mm was substantially larger at peri-implantitis (3.62 ± 3.72 cells/mm for implant A, 6.88 ± 5.73 cells/mm for implant B) than at periodontitis sites (0.74 ± 1.24 cells/mm). The difference in numbers of TRAP cells/mm between implant type B and teeth was statistically significant.

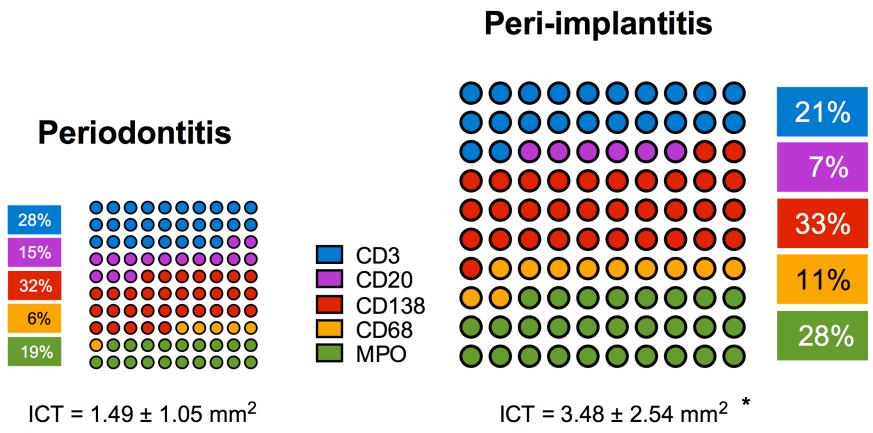
CD138-, CD68-positive cells and vascular structures (Study II)

The area proportions of the ICT that was occupied by CD138- and CD68-positive cells was significantly larger in peri-implantitis (13.24 ± 9.22 %, and 3.68 ± 3.53 %, respectively) than in periodontitis specimens (8.96 ± 6.71 %, and 2.13 ± 3.17 %, respectively). The density of vessels within the ICT was significantly larger in periodontitis (7.81 ± 5.09 %) than in peri-implantitis (2.75 ± 2.60 %). In the connective tissue portion lateral to the ICT, however, the proportion of vascular structures was significantly larger in peri-implantitis (8.58 ± 8.93 %) than in periodontitis (2.31 ± 2.34 %). In addition, the differences in vascular density between the two tissue compartments were statistically significant for both periodontitis and peri-implantitis specimens.

Total number of cells and cells/mm² (Study II)

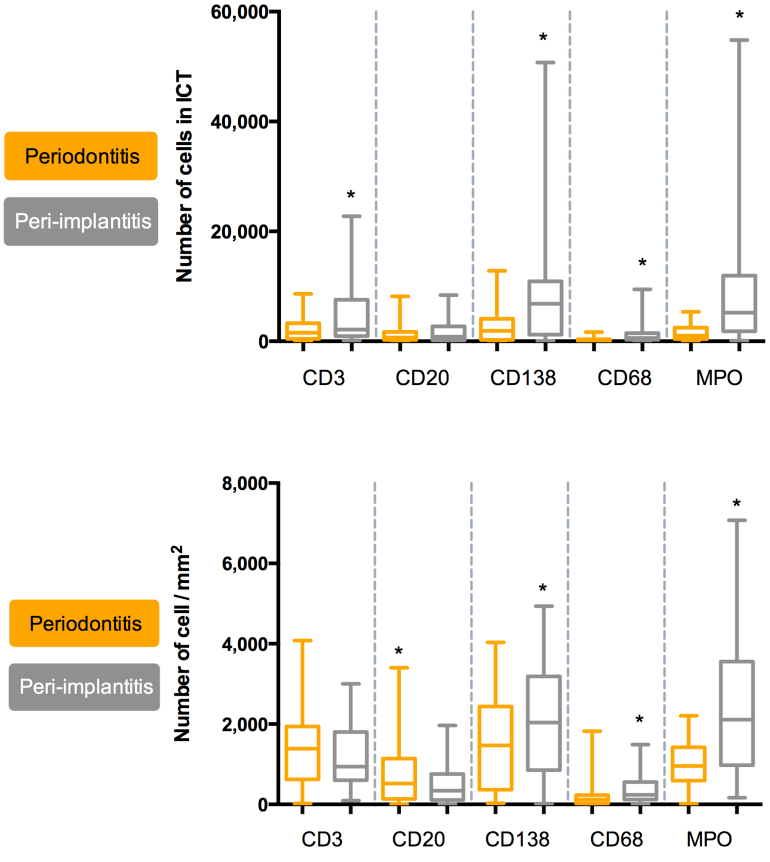
The percentage distribution of total number of cells in ICT of periodontitis and peri-implantitis lesions with the relative overall size of the ICT is presented in Figure 6. The large discrepancy on the overall size of the ICT between the 2 types of specimens is also illustrated in Figure 6.

*Figure 6. Percentage distribution of total number of cells in periodontitis and peri-implantitis lesions. (n=80) *p-value<0.05 between periodontitis and peri-implantitis lesions.*



The results from the assessments of cell size, the calculated total number positive cells, and number of cells/mm^2 within the ICT are illustrated in Figure 7. The estimated total number of inflammatory cells within ICT was significantly larger in peri-implantitis than in periodontitis sections. The numbers of CD3-, CD138-, CD68-, and MPO-positive cells were significantly larger in peri-implantitis than in periodontitis lesions.

*Figure 7. Total estimated number and density of positive cells in the ICT of periodontitis (n=40) and peri-implantitis (n=40) sites. *p-value<0.05 between periodontitis and peri-implantitis lesions.*



The overall density of inflammatory cells within the ICT (i.e., the number of cells/mm^2) was significantly higher in peri-implantitis than in periodontitis specimens. Specifically, the densities of CD138-, CD68-, and MPO-positive cells were significantly higher in peri-implantitis than in periodontitis lesions, whereas an opposite association was observed for CD20-positive cells.

The largest total number of cells or cells/mm^2 among the different phenotypes was found for MPO- and CD138-positive cells in peri-implantitis lesions. These two cell categories in peri-implantitis not only occurred in 3- to 6-times larger numbers than their counterparts in periodontitis lesions but also outnumbered other cell groups in both types of lesions.

The ANCOVA analysis of patient characteristics revealed that differences in the distribution of gender, age and smokers between the periodontitis and the peri-implantitis groups did not influence the results from the immunohistochemical assessment.

Treatment of peri-implantitis (Study III and IV)

Radiological findings

Preparatory period of ligature-induced breakdown (Study III)

The amount of bone loss that occurred during the preparatory period of ligature-induced breakdown varied between 3.57 and 3.73mm. (Table 10).

Table 10. Radiographical bone level alterations during the preparatory period prior to treatment. Mean values (±S.D.)

	Implant A	Implant B	Implant C	Implant D
Bone level changes during the preparatory period before surgical treatment (mm)	-3.58 (±0.76)	-3.72 (±0.65)	-3.73 (±0.47)	-3.57 (±0.63)

Period after surgical treatment of peri-implantitis (Study III and IV)

Three months after the peri-implantitis surgery, one implant B representing the test group was lost and the radiologic bone loss around this implant was assessed to the apical extension of the implant. The results from the radiological assessments are presented in Table 11.

In study III, in the control group (saline), radiographic bone gain was observed after surgical treatment at implants of type A and type C while additional bone loss was observed at implants of type B and type D. Bone loss at implant type D was significantly larger than at implant types A, B and C. In the test group (chlorhexidine), only implants of type C presented radiographic bone gain during the corresponding period, while additional bone loss was observed at implants of types A , B and D. The radiological analysis failed to demonstrate statistically significant differences between test and control procedures.

In study IV, bone gain was observed at implants in patients of groups 1 and 2, while additional bone loss was noted in the other two groups.

Table 11. Results from radiological examination after surgical treatment. Mean values (±S.D.)

Study III		All implants	Implant A	Implant B	Implant C	Implant D
Bone level changes between 2 weeks and 6 months after surgery (mm)	Control (saline)	- 0.52 (±2.09)	0.37 (±2.02)	- 0.20 (±1.88)	0.51 (±1.24)	- 2.77 (±1.58) *
	Test (chx)	- 0.27 (±1.85)	- 0.46 (±1.39)	- 0.18 (±2.64)	0.73 (±0.81)	- 1.15 (±2.01)
Study IV		All groups	Group 1 (AB+/AS+)	Group 2 (AB+/AS-)	Group 3 (AB-/AS+)	Group 4 (AB-/AS-)
Bone level changes between 2 weeks and 12 months after surgery (mm)		-0.21 (±1.32)	0.18 (±1.15) §	0.51 (±0.84) §	- 0.69 (±1.32) §	- 0.96 (±1.42) §

* *p*-value <0.05 implant D vs. implants A, B and C; § *p*-value <0.05 Groups 1 and 2 vs. Groups 3 and 4

Clinical findings

Study III

One implant B representing the test group was lost three months after the peri-implantitis surgery. During the period following surgical therapy clinical signs of inflammation in the peri-implant mucosa gradually resolved and towards the end of the experiment the majority of sites demonstrated absence of clinical signs of inflammation. At implants type D of the control group (saline), however, swelling and redness persisted in the peri-implant mucosa.

Study IV

Three patients (2 patients in group 3 and 1 patient in group 4) did not attend the examination at 6 months after surgery but attended the final examination (12 months). One patient with one affected implant and representing group 3, did not attend the examination at 6 and 12 months. All patients in groups 1 and 2 reported complete adhesion to the systemic antibiotic regimen. Five of these patients reported mild gastrointestinal problems. During the 1-year follow-up period, 6 implants in 6 patients were found to be disintegrated and, hence, removed (group 1: 1 implant/1 patient, group 3: 3 implants/3 patients, and group 4: 2 implants/2 patients). All lost implants had a modified surface.

The results from the clinical assessments are presented in Table 12. Reduction in PPD occurred in all treatment groups but was significantly larger in group 2 than in groups 3 and 4 at the 1-year examination. At 6 months following the surgical treatment of peri-implantitis, BoP remained at 53 % of affected implants. Further improvement (42%) was observed at 12 months, with no significant differences between treatment groups. At 12 months, SoP was observed at 18 % of all sites (Figure 8).

Table 12. Results from clinical examinations. Baseline (n=179) and changes at 6 (n=174) and 12 months (n=172) after surgical treatment. Mean values (±S.D.)

		All groups	Group 1 (AB+/AS+)	Group 2 (AB+/AS-)	Group 3 (AB-/AS+)	Group 4 (AB-/AS-)
Baseline probing pocket depth, at deepest site (mm)		7.82 (±1.52)	7.85 (±1.57)	7.93 (±1.50)	7.79 (±1.69)	7.78 (±1.25)
Probing depth changes (mm)	Baseline to 6 months	-2.71 (±1.71)	-3.03 (±1.58) #	-3.49 (±1.54) †	-2.18 (±1.54) †	-1.95 (±1.81) #†
	Baseline to 1 year	-2.58 (±1.97)	-2.80 (±1.87)	-3.44 (±1.66) †	-2.16 (±1.79) †	-1.69 (±2.22) †

p-value<0.05 Group 1 vs. Group 4; † *p*-value<0.05 Group 2 vs. Groups 3 and 4.

Treatment success was obtained at 45 % of all implants at 12 months after surgical therapy. The corresponding value assessed at the patient level was 38 % (Figure 9). The results from the analysis of treatment success indicated different outcomes between implant surface categories. Thus, treatment success was obtained overall in 79.1 % of implants and in 66.7 % of patients representing implant surface category A (non-modified surface). The corresponding data for implants with modified surfaces (categories B, C, D, E and F) were 34.1 % and 32.5 %, respectively. In addition, the absence of the adjunctive use of systemic antibiotics or local antiseptics had minor effect on treatment success for implant category A. In implant category B, however, no cases exhibited treatment success in the absence of systemic antibiotics (treatment groups 3 and 4).

The results from the logistic regression analysis are shown in Table 13.

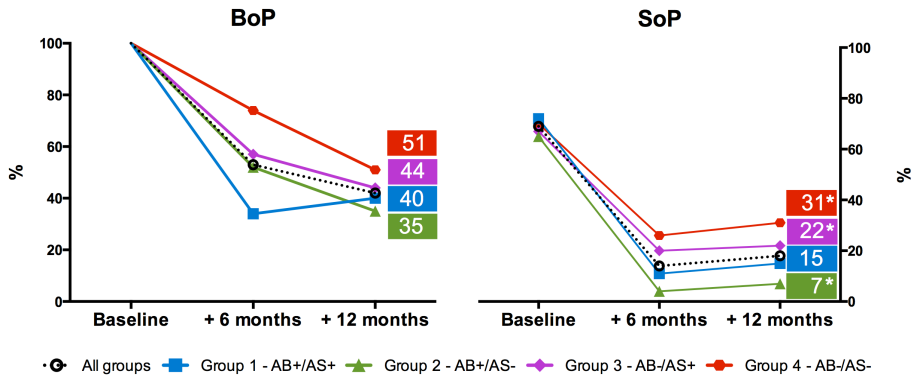
Table 13. Factors associated with treatment success: logistic regression analysis.

		OR	CI (95%)	*p*
Antibiotics	No	1	-	-
	Yes	0.55	0.11 - 2.72	0.462
Antiseptics	No	1	-	-
	Yes	0.634	0.30 - 1.32	0.221
CVD-related drug therapy	No	1	-	-
	Yes	0.21	0.09 - 0.48	<0.001
Implant surface modification	Non-modified	1	-	-
	Modified	0.032	0.01 - 0-115	<0.001
Interaction	Antibiotics (Yes) *x* Implant surface modification (Modified)	15.1	2.37 - 95.7	<0.001

CVD : Cardiovascular Diseases

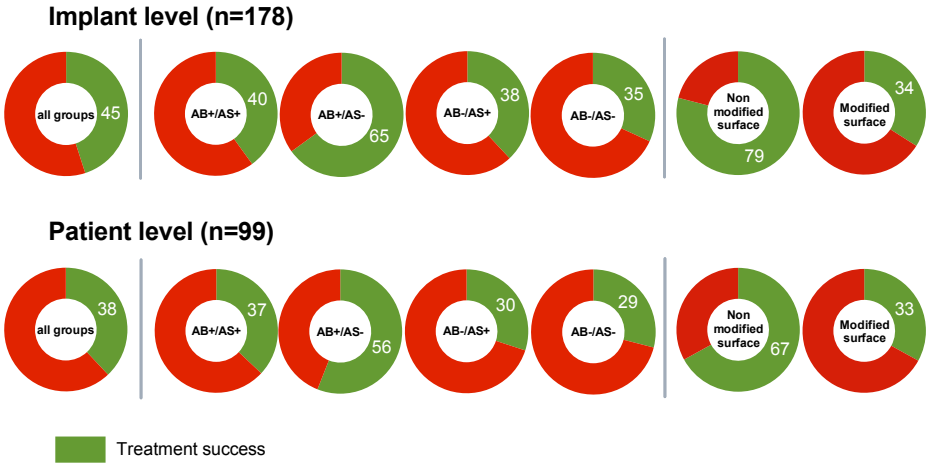
The adjunctive use of systemic antibiotics and local antiseptics had no impact on treatment success (OR 0.55; p=0.46 and OR 0.63; p=0.22 respectively), while CVD-related drug therapy had a negative effect (OR 0.21; p<0.0001).

Figure 8. Proportions of implants exhibiting BoP and SoP (%) at baseline (n=179), at 6 (n=174) and 1 year (n=172) after surgical treatment.



** p-value <0.05 Group 2 vs. Groups 3 and 4.*

Figure 9. Proportions (%) of treatment success at implant level (n=178) and patient level (n=99).



Using implant with a non-modified surface (category A) as a reference, implants with modified surfaces (categories B, C, D, E and F) showed a significantly lower OR for treatment success (OR 0.032; $p<0.0001$). Interaction between the use of antibiotics and surface characteristics was observed in the data analysis, indicating a positive effect of the adjunctive use of systemic antibiotics in treatment of peri-implantitis around implants with modified surfaces (OR 15.1; $p=0.004$).

Histological findings (Study III)

Gross observations

At control sites, the peri-implant mucosa around implants A and C exhibited a thin barrier epithelium, apical of which a non-inflamed connective tissue was facing the implant surface. Scattered inflammatory cells were occasionally found in the marginal portion of the connective tissue around the implants A and C. The majority of control specimens representing implant B exhibited clusters of inflammatory cells of varying size in the marginal portion of the peri-implant connective tissue. All implants D exhibited no signs of resolution of peri-implantitis characterized with an extensive osseous defects and a large inflammatory cell infiltrates in the surrounding connective tissue.

At test sites, the peri-implant mucosa around implants B and C exhibited a barrier epithelium of varying length, apical of which a fibrotic connective tissue portion was observed, the majority of specimens representing implant A and D presented with inflammatory cells residing in the connective tissue compartment lateral and apical to the barrier/pocket epithelium.

Histometric measurements

Among the control group specimens, the residual bony defect area at implants of type D was significantly larger than that of implants A, B and C (Figure 10).

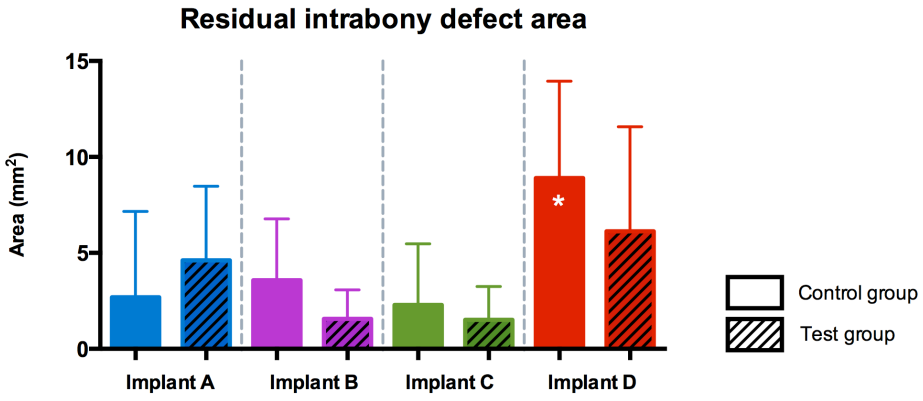
The overall distribution of the ICT scores differed between the test and control groups (Figure 11). While in implants B, C and D the test procedure resulted in lower scores than the control procedure, a reverse relationship was found for implants A. Marked differences in score distribution were also detected between the implant types. Thus, in the test group 5 out of 6 implants of type C and 4 out of 6 implants of type B exhibited an ICT score 0, whereas the majority of implants of type A and D presented with a score 3. In the control group the largest proportion of implants with score 0 was found among implants A, while 83 % of implants D had an ICT score 3.

Microbiological findings (Study III, IV)

Study III

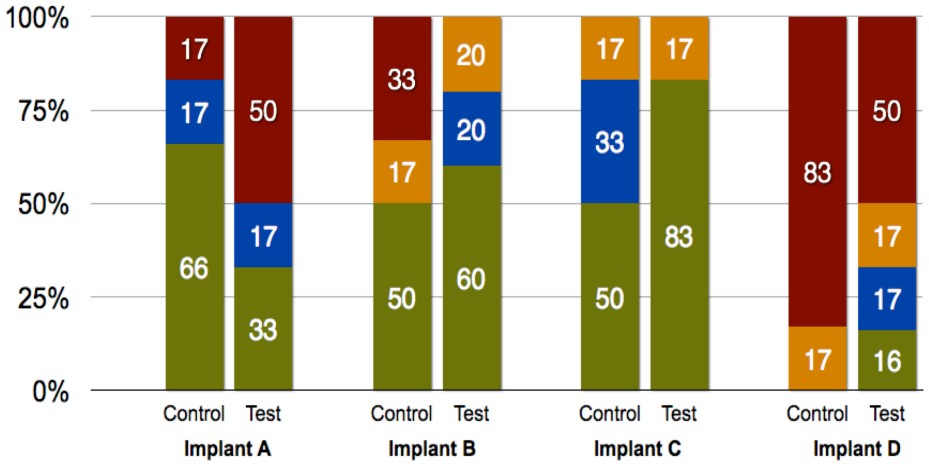
In terms of total count of bacteria, no statistically significant differences were observed among implants prior to surgery. The total count, however, had decreased significantly at 3 and 5 months after surgery in both test and control groups, except for implants D. An increase of the total DNA-probe counts occurred at implant D of the control groups (Table 14).

Figure 10. Residual intrabony defect area representing control (saline) and test (chlorhexidine) procedures for implants type A, B, C, D. (n=6)



* *p*-value <0.05 between implant D versus implant A, B and C of the control group.

Figure 11. ICT score for control (saline) and test (chlorhexidine) sites at implant type A, B, C, D. Score 0 (green), 1 (blue), 2 (orange), 3 (brown).



Statistically significant differences in DNA-probe counts were observed between implant C and D both at 3 and 5 months. No statistically significant differences were found between test and control sites for any of the implant types.

Table 14. Changes in total DNA-probe counts (x10^5) at control (saline) and test (chlorhexidine) groups for each implant type from surgery to 3 and 5 months after surgery. Mean values (S.D.) (n = 6)

Total DNA-probe counts changes (x10^5)	Implant A		Implant B		Implant C		Implant D	
	Control (saline)	Test (Chx)	Control (saline)	Test (Chx)	Control (saline)	Test (Chx)	Control (saline)	Test (Chx)
Day of surgery - 3 months after surgery	-4.77 *	-4.49 *	-9.99 *	-6.93 *	-10.4 *	-15 *	7.46	-6.54
Day of surgery - 5 months after surgery	-5.8 *	-9.97 *	-10.83 *	-11.69 *	-12.6 *	-14.9 *	5.23	-3.47

**: p-value <0.05 between baseline versus 3 and 5 months for implant A, B, C*

Study IV

The results microbiological analysis are reported in Figure 12. The overall profile of changes in total DNA counts was similar for the 4 treatment protocols and exhibited a significant decline during the 12-month period after surgical therapy. The total viable counts also decreased after surgery in all treatment groups.

Checkerboard and culture analysis showed that *Fusobacterium nucleatum* and *Prevotella intermedia/nigrescens* were the most common type of bacteria presenting moderately heavy/heavy growth at baseline (71 % and 46 % of the patients, respectively) and 1 year after surgical treatment (54 % and 43 % of the patients, respectively). Moderately heavy/heavy growth of *Staphylococcus aureus* was detected in one patient before surgery, but never at the 1-year examination. No patient presented with moderately heavy/heavy growth of *Aggregatibacter actinomycetemcomitans*. Detailed data from checkerboard and culture analysis are presented in Table 15.

Figure 12. Mean total DNA-probe counts changes (x10^5) and mean Total Viable Counts changes (x10^7) after surgical treatment of peri-implantitis for each treatment group. Significant decrease of total DNA-probe counts after surgery in all treatment groups.

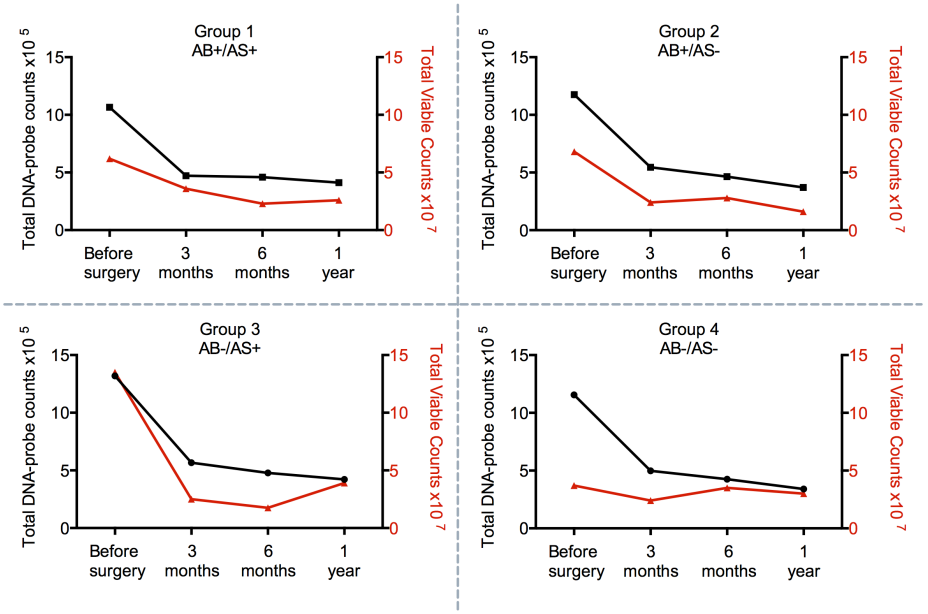


Table 15. Percentage of patients with not detected and detected bacteria (by checkerboard/culture analysis) before and 1 year after surgical treatment.

DNA-DNA checkerboard				Species	Culture			
Signal > 10^5 bacteria (score 3, 4, 5)		No detected signal (score 0)			Not detected (score 0)		Detected in moderately heavy/ heavy amounts (score 3, 4)	
Before surgery	1 year	Before surgery	1 year		Before surgery	1 year	Before surgery	1 year
0	0	92	94	**A.a**	100	98	0	0
0	1	41	48	**C.rectus**	54	63	30	22
73	36	0	2	**F.nucleatum**	17	31	71	54
7	3	74	77	**P.gingivalis**	87	91	10	7
48	19	33	60	**P.intermedia/ P.nigrescens**	37	39	46	43
				S.aureus	97	100	1	0
				S.epidermidis	80	75	0	5
				Enterococci	96	98	2	2
				AGNB	88	82	10	18
1	0	78	75	**F.alocis**				
4	4	42	48	**P.endodontalis**				
3	2	6	12	**P.micra**				
1	1	69	61	**P.tannerae**				
2	2	45	50	**T.denticola**				
4	4	31	40	**T.forsythia**				

AGNB : Aerobic Gram-negative bacilli

Main findings

- Spontaneous progression of experimental peri-implantitis resulted in greater amount of bone loss, larger inflammatory cell infiltrates with larger proportions of neutrophil granulocytes and osteoclasts than experimental periodontitis. (**Study I**)

- Human peri-implantitis lesions were more than twice as large and contained significantly larger area proportions, numbers and densities of CD138-, CD68- and MPO-positive cells than human periodontitis lesions. (**Study II**)

- The local use of chlorhexidine has minor influence on resolution of peri-implantitis following surgical treatment. (**Study III**)

- Implant surface characteristics influence treatment outcomes. (**Study III** and **IV**)

- The adjunctive use of systemic antibiotics increased the probability for treatment success at implants with modified surfaces but not at implants with a non-modified surface. (**Study IV**)

Concluding remarks

The current series of studies employed a translational approach in the comparison between peri-implantitis and periodontitis lesions and the evaluation of surgical treatment of peri-implantitis.

Translational research

Translational research is an important aspect of research, bringing together findings from pre-clinical *in vivo* studies to subsequent clinical implementation. Thus, when adequately designed and conducted, pre-clinical *in vivo* research provides important information that adds to our understanding in the pathogenesis and treatment of peri-implantitis. The dog experiments used in this series of experiments are suitable pre-clinical models to study peri-implantitis. Dogs exhibit a natural susceptibility to periodontal disease (Gad, 1968; Lindhe et al., 1973, 1975; Kortegaard et al., 2008) and jaw bone anatomy in dogs allows the placement of commercially available dental implants (Grunder et al., 1993; Wetzel et al., 1999; Nociti Júnior et al., 2001; Shibli et al., 2003; Albouy et al., 2008; Schwarz et al., 2011).

While studies using animal models are an important part of dental research, the translation of results into therapeutic strategies for humans is far from straightforward. The validity of an animal model is judged in terms of similarities between the model and the human condition to be studied. Thus, an animal model is considered as valid in the presence of similarities with the human condition in terms of aetiology, physiopathology and response to therapeutic interventions (Bhogal & Balls, 2008). Evidence of validity is usually divided into three aspects: *predictive validity* (effective interventions in the animal model demonstrate a similar effect clinically), *face validity* (similarities in pathogenesis between the disease in the animal model and the human condition) and *construct validity* (a factor evaluated in the experiment has a similar role in the disease model as in the clinical situation) (Denayer et al., 2014).

Pathogenesis of peri-implantitis

An analysis of the pathogenesis of peri-implantitis and periodontitis in humans has its limitations. The biopsy-sampling procedure should ideally include the harvesting of the entire lesion together with the supra-crestal soft tissue portion and the crestal bone. From an ethical point of view, sampling of human biopsies is often restricted to the soft tissue component, as the supporting bone can not be retrieved. Animal models have been used

in pre-clinical *in vivo* studies, providing access to the entire disease process, including both soft and hard tissues.

Study I demonstrated that more bone loss occurred at peri-implantitis than at periodontitis sites during the period following ligature removal. The histological analysis revealed that peri-implantitis specimens exhibited lesions that were larger, extended closer to the bone crest and contained larger proportions of neutrophil granulocytes and osteoclasts than periodontitis lesions. The radiological and histological findings presented in *study I* are in agreement with observations made by Lindhe et al. (1992). Cotton ligatures were placed around teeth and implants in five beagle dogs and plaque was allowed to accumulate. While the ligatures were removed after 6 weeks, plaque formation continued and after an additional 4-week period clinical and radiological examinations were performed and block biopsies were obtained. It was reported that clinical signs of inflammation and radiographic bone loss were more pronounced at peri-implantitis than at periodontitis sites. Similar findings were presented by Schou et al. (1993), who compared a 7-week period of ligature-induced breakdown around implants as well as ankylosed and non-ankylosed teeth in monkeys. The authors reported that bone loss was more pronounced around implants than teeth and that bone loss was associated with a high number of osteoclasts in the histological specimens. While Schou et al. (1993) and Lindhe et al. (1992) studied lesions in peri-implant and periodontal tissues resulting from subgingival plaque formation in the presence of cotton ligature and one month after ligature removal, the experiment in *study I* applied the modified ligature-model introduced by Zitzmann et al. (2004) and tissue reactions to plaque formation were analyzed at 6 months following the removal of ligatures.

While quantitative analysis of experimentally induced disease was performed in *study I*, qualitative evaluations of cells involved in human peri-implantitis and periodontitis lesion were addressed in *study II*. Thus, the analyses of human specimens in *study II* demonstrated that peri-implantitis lesions were more than twice as large and contained significantly larger area proportions, numbers and densities of CD138 (plasma cells)-, CD68 (macrophages)- and MPO (neutrophiles granulocytes)-positive cells than periodontitis lesions. The findings on differences in size of the lesions between the two conditions reported are in agreement with results from *study I*, thus pointing to the validity of the experimental model. There are few reports on human peri-implantitis lesions. Sanz et al. (1991) analyzed soft tissue biopsies from 6 patients with peri-implantitis and reported that about 2/3 of the connective tissue portion of the biopsies were occupied by an infiltrate consisting of plasma cells, mononuclear cells and enlarged blood vessels. Berglundh et al. (2004) analyzed soft tissue biopsies obtained from 12 implant sites with severe peri-implantitis in 6 patients. The histological analysis demonstrated that the lesions occupied almost the entire connective tissue compartment and extended apical to the pocket epithelium. It was also observed that the lesions contained not only plasma cells and lymphocytes but also PMN

cells in high numbers, which were residing in peri-vascular compartments distant from the "pocket area". These data are consistent with results obtained both in *study II* and *study I*.

The examination of the two types of lesions in *study II* is relevant in regards to similar appraisals of differences between lesions in varying forms of periodontal diseases. Thorbert-Mros et al. (2014) analyzed gingival biopsies from patients with either severe generalized periodontitis or longstanding gingivitis. It was reported that periodontitis lesions were twice as large and contained significantly larger densities of cells positive for the markers CD138 and CD68 than gingivitis lesions. The authors concluded that the large number and high density of plasma cells were the hallmarks of advanced periodontitis lesions and the most conspicuous difference in relation to longstanding gingivitis lesions. Gualini & Berglundh (2003) evaluated differences between peri-implant mucositis and peri-implantitis lesions. The authors examined immunohistochemical characteristics of soft tissue biopsies obtained from 16 patients and reported that peri-implantitis lesions contained significantly greater proportions of B cells and elastase-positive cells (indicating PMN cells) than mucositis lesions. Thus, the severity of a condition appears to correlate with the size of the lesion and a cell profile with enhanced densities and numbers of the B-cell /plasma cell line together with neutrophil granulocytes and macrophages. Peri-implantitis lesions carry such characteristics.

Considering differences in numbers and densities of CD138-, CD68-, and MPO-positive cells between peri-implantitis and periodontitis lesions, it was emphasized in *study II* that the inflammatory response at peri-implantitis sites was stronger by promoting cells, which are part of both the innate and the adaptive host response. Studies on gene expression of pro-inflammatory markers at periodontitis and peri-implantitis sites (Venza et al.,2010; Becker et al.,2014) presented similar findings. However, it should be noted that the analyses performed by Venza et al. (2010) and Becker et al. (2014) were not restricted to the inflammatory lesions as the processing included the entire soft tissues biopsy.

Treatment of peri-implantitis

A review of the current literature reveals that many pre-clinical *in vivo* experiments and clinical studies have been performed on the treatment of peri-implantitis. However, as reported by Faggion et al. (2011) and Graziani et al. (2012), there is a large variation among clinical studies in terms of design (case series, controlled clinical trials, randomized control trials), sample size (ranging from 9 to 45 patients), follow-up (ranging from 3 months to 4 years) and type of intervention (different decontamination procedures and/or bone augmentation procedures). Moreover, Claffey et al. (2008) concluded in a review that access surgery combined with implant surface decontamination for treatment of peri-implantitis had rarely been investigated in a controlled manner. The authors also reported

that a great variation existed in terms of use and regimen of systemic antibiotics (alone or in combination with other antimicrobial agents) both in pre-clinical *in vivo* and clinical studies. Adjunctive systemic antibiotics has been used in many clinical trials (Behneke et al., 2000; Leonhardt et al., 2003; Romeo et al., 2005, 2007; Roos Jansåker et al., 2007, 2011, 2014; Roccuzzo et al., 2011; Serino & Turri, 2011; Aghazadeh et al., 2012; Heitz-Mayfield et al., 2012; Wiltfang et al., 2012; Serino et al., 2014;), but no study evaluated their adjunctive benefit. As resolution of peri-implantitis following surgical therapy without adjunctive use of systemic antibiotics has been demonstrated in pre-clinical *in vivo* studies (Schwarz et al., 2006; Shibli et al., 2006, Albouy et al., 2011), randomized and controlled clinical trials in patients with peri-implantitis are ethically justified. At the 8th European Workshop of Periodontology, Sanz & Chapple (2012) emphasized the need for parallel-arm randomized controlled studies, including a large sample size and at least 1 year follow-up, for evaluating the adjunctive effect of systemic antibiotics on surgical treatment of peri-implantitis. Similar statement were made in a consensus report on prevention and management of biologic and technical implant complications (Heitz-Mayfield & Mombelli, 2014). *Study IV* reports on a 1-year follow-up of 100 patients enrolled in a prospective randomized controlled clinical trial, designed to investigate the effect of adjunctive systemic antibiotics on surgical treatment of peri-implantitis. As recommended by Sanz & Chapple (2012), treatment success were defined using a composite outcome of disease resolution, including PPD ≤ 5mm, absence of bleeding/suppuration at the 12-month examination and bone loss ≤ 0.5mm between 2 weeks and 12 months after surgical therapy.

Conclusions regarding the influence of implant surface characteristics on treatment outcome of surgical therapy of peri-implantitis revealed in *study IV* validate observations made in the pre-clinical *study III*. Results from the longitudinal assessments of bone level changes in radiographs as well as microbiological and histological analyses in *study III* demonstrated lower occurrence of resolution of peri-implantitis at implants with a TiUnite surface (corresponding to implants of type D) when compared to implants with TiOblast, Osseospeed and AT-1 surfaces. This observation was confirmed by the results reported in *study IV* where implants with a TiUnite surface (corresponding to implants of category B in *study IV*) exhibited the smallest overall frequency of treatment success. Albouy et al. (2011), in a pre-clinical experiment and Roccuzzo et al. (2011) in clinical study also concluded that treatment outcomes of surgical therapy of peri-implantitis were influenced by implant surface characteristics. Albouy et al. (2011) examined radiologic and histological outcomes following surgical treatment of peri-implantitis in dogs. Experimental peri-implantitis was induced around different types of implants (Turned, SLA, TiOblast and TiUnite). Surgical therapy included mechanical cleaning of implants and was performed without using adjunctive systemic antibiotics or local antiseptics. Resolution of inflammation as observed in histological analysis was obtained from implants with non-modified and with TiOblast surfaces. In addition, the assessments of bone level changes in

radiographs during the 6-month healing period revealed bone gain at implants with non-modified surfaces and at two of the implant categories with modified surfaces (TiOblast and SLA), whereas bone loss occurred at implants with a TiUnite surface. Roccuzzo et al. (2011) evaluated the treatment of peri-implantitis around implants with either a rough (TPS) or a moderately rough (SLA) surface in 26 patients. One year follow-up demonstrated that the surgical therapy was more effective in reducing PPD, BoP and bone defects at implants with moderately rough surfaces.

The differences in resolution of peri-implantitis lesions at different implant types observed in *study III* and *IV* might be related to the difficulties of decontaminating exposed implant surfaces. A number of different decontamination protocols including the use of chemical agents, air-abrasives or lasers, have been presented in pre-clinical *in vivo* studies and clinical trials. Gauzes soaked in chlorhexidine or saline were commonly used and the two detergents were applied either alone or in combination. Wetzel et al. (1999) in a dog study, analyzed treatment of experimental peri-implantitis using 0.12 % solution of chlorhexidine digluconate to decontaminate implant surfaces and reported that bone fill occurred in the osseous defects around all types of implants following therapy. In a dog study aiming to evaluate differences in bone fill and re-osseointegration at implants with 2 different surfaces, Persson et al. (2001) reported resolution of peri-implantitis lesions following the local use of pellets soaked in saline at both types of implants. Similar results were reported in a study performed in dogs by You et al. (2007), who combined both chlorhexidine and saline in the cleaning of implant surfaces. These findings are in agreement with *study III,* which failed to demonstrate that chlorhexidine had any major effect on treatment outcomes but reported that resolution of peri-implantitis following surgical treatment was possible by using a gauze soaked in saline to decontaminate implant surfaces. The observed lack of benefit of the local use of chlorhexidine on treatment outcome reported in *study III* is validated by findings made in *study IV*. In a randomized controlled clinical study with 1, 2 and 4 years follow-up, Schwarz et al. (2011, 2012, 2013) evaluated the impact of two surface decontamination methods (Er-YAG laser versus plastic curets + cotton pellets soaked in sterile saline) on the clinical outcomes of combined surgical treatment of peri-implantitis. Both treatment regimens resulted in similar and statistically significant short-term clinical improvement and radiographic bone fill. After a follow-up period of 2 and 4 years, the authors concluded that treatment outcomes in surgical therapy of advanced peri-implantitis were not influenced by the method of surface decontamination. De Waal et al. (2013) evaluated in a randomized, double-blind, placebo-controlled trial the effect of implant surface decontamination with chlorhexidine/cetylpyridinium chloride on microbiological and clinical parameters. Thirty patients (79 implants) with peri-implantitis were treated with resective surgical treatment. The use of the combined detergents resulted in greater immediate suppression of anaerobic bacteria

than the placebo procedure, but did not result in superior clinical outcomes at 1 year. These findings partly confirm data presented in the *study III* and *IV*.

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Appendix

I. **Carcuac O.**, Abrahamsson I., Albouy JP., Linder E., Larsson L., Berglundh T. (2013) Experimental periodontitis and peri-implantitis in dogs. *Clinical Oral Implant Research* **24**, 363-371

II. **Carcuac O.**, Berglundh T. (2014) Composition of human periodontitis and peri-implantitis lesions. *Journal of Dental Research* **93(11)**, 1083-1088

III. **Carcuac O.**, Abrahamsson I., Charalampakis G., Berglundh T. (2015) The effect of the local use of chlorhexidine in surgical treatment of experimental peri-implantitis in dogs. *Journal of Clinical Periodontology* doi: 10.1111/jcpe.12332 [Epub ahead of print]

IV. **Carcuac O.**, Derks J., Abrahamsson I., Charalampakis G., Wennström JL., Berglundh T. (2015) Adjunctive systemic antibiotics enhance treatment outcomes of surgical therapy of peri-implantitis at implants with modified surfaces but not at implants with non-modified surfaces. A randomized controlled clinical trial. *In manuscript.*

CLINICAL ORAL IMPLANTS RESEARCH

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Experimental periodontitis and peri-implantitis in dogs

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Key words: animal experiment, biopsy, bone, dental implant, histology, inflammation, lesion, peri-implant disease, periodontal disease, radiology

Abstract

Aim: To analyze the tissue reactions following ligature removal in experimental periodontitis and peri-implantitis in dogs.

Material and methods: Four implants with similar geometry and with two different surface characteristics (turned/TiUnite Nobel BioCare AB, Göteborg) were placed pair-wise in a randomized order in the right side of the mandible 3 months after tooth extraction in 5 dogs. Experimental peri-implantitis and periodontitis were initiated 3 months later by ligature placement around implants and mandibular premolars and plaque formation. The ligatures were removed after 10 weeks, and block biopsies were obtained and prepared for histological analysis 6 months later.

Results: It was demonstrated that the amount of bone loss that occurred during the period following ligature removal was significantly larger at implants with a modified surface than at implants with a turned surface and at teeth. The histological analysis revealed that peri-implantitis sites exhibited inflammatory cell infiltrates that were larger, extended closer to the bone crest and contained larger proportions of neutrophil granulocytes and osteoclasts than in periodontitis.

Conclusion: It is suggested that lesions produced in experimental periodontitis, and peri-implantitis are different and that implant surface characteristics influence the inflammatory process in experimental peri-implantitis and the magnitude of the resulting tissue destruction.

Peri-implantitis is characterized by inflammation in peri-implant tissues and loss of supporting bone (Zitzmann & Berglundh 2008) and has many clinical features in common with its counterpart around teeth. Clinical diagnosis of the condition includes the assessment of Bleeding on Probing and radiological signs of bone loss. Pus is also a common finding in peri-implantitis sites (Lang & Berglundh 2011).

While clinical characteristics of peri-implantitis may resemble those of periodontitis, histopathological features of the two types of lesions appear to present with large differences. In a review performed in conjunction with the 7th European Workshop on Periodontology Berglundh et al. (2011) appraised information on peri-implantitis and periodontitis lesions. It was reported that few studies evaluated peri-implantitis in human biopsy material, while comprehensive information was available regarding human periodontitis lesions. Similarly, few experimental studies comparing peri-implantitis and periodontitis lesions were accessible (Lindhe et al. 1992; Lang et al. 1993; Schou et al. 1993; Nociti et al. 2001). Most experimental studies on peri-implantitis employed the ligature model to induce breakdown of peri-implant soft and hard tissues. This model has been extensively used in studies on experimental periodontitis and was introduced to promote tissue breakdown in short time as earlier studies on the natural development of periodontitis in dogs demonstrated that signs of the disease with attachment and bone loss occurred after several years (Lindhe et al. 1973, 1975; Hamp & Lindberg 1977). Thus, ligatures were used together with plaque formation to initiate and maintain a pathological process in gingival tissues (Kennedy & Polson 1973). The placement of a ligature in

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a subgingival position disrupts the soft tissue seal around teeth and implants and opens the pocket for biofilm formation. While a ligature made of cotton or silk may not induce bone loss by itself, the developing inflammatory process in the connective tissue that results from the biofilm formation mediates tissue destruction during the experiment. The early response to ligature placement and biofilm formation in experimental periodontitis was described in a study in monkeys (Heijl et al. 1976), and it was understood that tissue breakdown faded over time and that ligatures had to be removed and replaced to promote continuous tissue destruction.

In most studies on experimental periodontitis, the ligatures were removed about one month prior to biopsy to allow resolution from an acute to a chronic process. Using a similar procedure in experimental peri-implantitis, results indicated that the resolution observed in experimental periodontitis sites did not occur one month after ligature removal around implants (Lindhe et al. 1992). On the contrary, previous experiments from our laboratory have pointed to the finding of a continuing destructive process also after the removal of ligatures in experimental peri-implantitis (Zitzmann et al. 2004; Berglundh et al. 2007; Albouy et al. 2008, 2012), whereas similar observations in experimental periodontitis have not been made. The aim of the present study was to analyze the tissue reactions following ligature removal in experimental periodontitis and peri-implantitis in dogs.

Material and methods

Animals

The study protocol was approved by the regional Ethical Committee for Animal Research, Göteborg, Sweden. Six 16-month-old Labrador dogs (3 females; weight 20 kg, 3 males; weight 30 kg) were used. The outline of the experiment is presented in Fig. 1. During all surgical procedures, general anesthesia was induced with intravenously injected Propofol (10 mg/ml, 0.6 ml/kg) and sustained with $N_2O : O_2$ (1 : 1.5–2) and Isoflurane employing endo-tracheal intubation.

Implant placement

The mandibular premolars and the first molar and the three anterior premolars of the maxilla were extracted on the right side in all dogs. Three months later, mucoperiosteal flaps were elevated, and 4 implants with similar geometry and with two different surface characteristics (MKIII NP, 3.3 × 10 mm; Nobel BioCare AB, Göteborg, Sweden/ implant group A; turned surface and implant group B; TiUnite surface) were placed pairwise in a randomized order in the edentulous premolar area in the mandible as reported previously (Albouy et al. 2012). The flaps were adjusted and sutured around healing abutments. The sutures were removed after 2 weeks, and a plaque control regimen, which called for tooth and abutment cleaning 3 times a week for 3 months, was initiated.

Experimental periodontitis and peri-implantitis

Three months after implant installation, experimental peri-implantitis and periodontitis were initiated. Plaque control procedures were abandoned, and cotton ligatures were placed in a subgingival position around the 4th, 3rd, and 2nd premolars in the left side of the mandible and in a corresponding position around the neck portion of the implants in the right side of the mandible in a manner previously described (Lindhe et al. 1992).

A set of radiographs was obtained from tooth and implant sites using a customized film holder (Kerr Hawe, Bioggio, Switzerland) as previously described by Persson et al. (1999) and Albouy et al. (2008, 2009). The radiographs were analyzed in an Olympus SZH10 stereo macroscope (Olympus optical co, GmbH, Hamburg, Germany), and digital images were obtained with a Leica DFC280 camera (Leica, GmbH, Wetzlar, Germany). The abutment-implant junction at implant sites and the cemento-enamel junction at tooth sites were used as reference landmarks for the radiologic measurements. The vertical distance between the reference landmark and the marginal bone level was assessed at the mesial and distal aspects of each implant/ tooth using the QWin software (Leica Qwin Standard V3.2.0; Leica Imaging Systems Ltd, Cambridge, UK). Double assessments were made by two examiners with a 2-month interval.

The ligatures were removed, and a new set of ligatures was placed in a more apical position at all sites after 3 weeks. The ligature shift procedure was repeated 3 weeks later and finally removed at 10 weeks after the initiation of the experimental breakdown procedure (baseline, Fig. 1). Plaque accumulation continued during the subsequent 26-week period, and radiographs were obtained at baseline, 10, 16, and 26 weeks after ligature removal.

Biopsy and histological preparation

Twenty-six weeks after ligature removal, the dogs were euthanized with a lethal dose of Sodium-Pentothal® (Hospira Enterprises B. V., Hoofddorp, Netherlands) and perfused through the carotid arteries with a fixative (4% formaldehyde). The mandibles were retrieved, and tissue blocks from tooth and implant sites were dissected using a diamond saw (Exakt, Kulzer, Norderstedt, Germany) and stored in the fixative. Two blocks were produced from the tooth site: One posterior block containing the 4th premolar and the distal root portion of the 3rd premolar, and one anterior block containing the 2nd premolar and the mesial root portion of the 3rd premolar. Tissue blocks were processed from each implant unit. Using a randomization protocol, 50% of the tissue blocks from tooth and implant sites were processed for ground sectioning according to the methods described by Donath & Breuner (1982) while the remaining samples were decalcified and embedded in paraffin (tooth sites) (Lindhe et al. 1992) or further prepared according to the "fracture technique" (implant sites) (Berglundh et al. 1994) and embedded in paraffin.

Ground sectioning

The tissue samples selected for ground sectioning were dehydrated in increasing grades of ethanol and embedded in Technovit 7200 VLC-resin (Kulzer, Friedrichsdorf, Germany) and prepared as described previously (Albouy et al. 2012). From each block (tooth and implant), 2 parallel sections were obtained in mesio-distal plane, and 2 parallel sections were obtained in a bucco-lingual plane. The sections were reduced by microgrinding (Exakt, Apparatebau, Norderstedt, Germany) to a final thickness of about 30 μm and

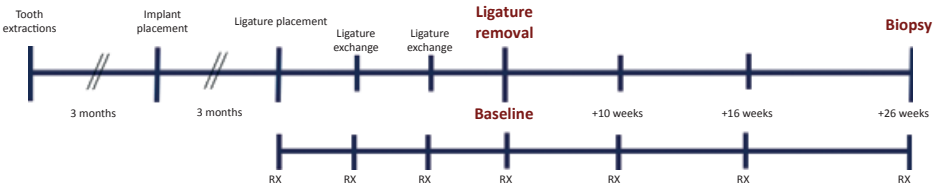

Fig. 1. Outline of the study.

stained in toluidine blue and fibrin stain of Ladewig (Donath & Breuner 1982).

Decalcified specimens

The tissue specimens were placed in EDTA. The tissue samples that included the implant and the surrounding soft and hard peri-implant tissues were processed using the fracture technique as described by Berglundh et al. (1994). In brief, incisions were made through the peri-implant tissues before the hard tissue was fully decalcified and 4 units, mesio-buccal, disto-buccal, mesio-lingual, and disto-lingual, were obtained and separated from the implant. Decalcification was completed in EDTA. The tooth sites were prepared when the decalcification process was completed. All tissue samples were embedded in paraffin, and 5 μm sections were produced. While sections from the implant units were produced parallel with the long axis of the implant, the tooth units were sectioned in a mesio-distal (P_2–P_3 or P_3–P_4) and a bucco-lingual plane (mesial root of P_2 or distal root of P_4).

Immunohistochemistry

Immunohistochemical preparation was performed in the paraffin-embedded sections. The panel of monoclonal antibodies that were used is presented in Table 1. The sections were de-waxed and incubated in antigen retrieval solution at 60°C over night. The DIVA antigen retrieval solution (Biocare medical, Concord, CA, USA) was used for antigen retrieval for staining with CD20, Myeloperoxidase (MPO) and IgG antibodies, while TE buffer was used for the CD3 antibody. The sections were incubated with primary antibodies for 30 min followed by incubation with MACH 4 ALP (Biocare medical) for 30 min. Positive cells were detected using the Vulcan Fast Red substrate (Biocare medical). The enzymatic activity of tartrate resistant acid phosphatase (TRAP; acid phosphatase, leukocyte kit, Sigma-Aldrich Inc, St. Louis, MO, USA) was used as a marker of osteoclasts.

Histometric analysis (ground sections)

The histological examinations were performed in a Leica DM-RBE microscope (Leica, Heidelberg, Germany) equipped with an image system (Q-500 MC; Leica, Wetzlar, Germany). In the ground sections, the following landmarks were identified and used for the linear measurement: the gingival/peri-implant mucosa margin (GM/PM), the abutment–fixture junction (A/F) at implant sites, the cemento-enamel junction (CEJ) at tooth sites, the apical termination of the biofilm (aPlaque) on the implant/tooth surface, the apical termination of the pocket epithelium (aPE), the marginal position of bone closest to the implant/tooth (B), the most coronal extension of the bone crest (BC), and the coronal and apical extension of the infiltrated connective tissue (cICT and aICT). The distance between the ICT and the lateral bone wall of the intra-bony defects (ICT-Bw) was measured in three locations; coronal, middle, and apical. The surface area of the ICT (area ICT) in the connective tissue was evaluated by outlining its circumference with a cursor.

Analysis of cell markers (paraffin sections)

The histological quantitative assessments of cell markers were performed using a microscope equipped with an image system (Leitz DM-RBE Q-500 MC® image system; Leica). An interference contrast setting at a magnification of ×400 was applied as previously described (Liljenberg et al. 1994; Zitzmann et al. 2001). A point counting procedure was used to determine the percentage of positive cell markers within the ICT. A lattice comprising 400 points was superimposed over the tissue area. Cross points that indicated the positive cell markers in the compartment to be examined were counted and related to the total counts for the entire ICT (%). TRAP-positive cells were analyzed with regard to the number cells found: (i) within a 200 μm-wide zone immediately lateral to the bone crest, and (ii) in contact with the bone crest. The number of TRAP-positive cells/mm was reported.

Data analysis

The SPSS 12.0 software package (SPSS Inc, Chicago, IL, USA) was used. Mean values for all variables were calculated for each implant/tooth unit in each animal as a basis for the statistical analysis. Using the animal as the statistical unit, differences were analyzed using analysis of variance (ANOVA) and the Student–Newman–Keuls test. A P-value <0.05 was considered as significant. A statistical package specially designed for multilevel modeling (MLwiN 2.02; Center for Multilevel Modelling at University of Bristol, Bristol, UK) was used to investigate the influence of dogs, implant/tooth, and site-related covariates on the outcome variables.

Table 1. **The panel of antibodies used for the immunohistochemical analysis**

Antibody (clone)	Specificity	Dilutions
CD3	T cells	1 : 200
CD20	B cells	1 : 800
MPO	Neutrophils, macrophages	1 : 1000
IgG	IgG-positive cells (plasma cell / B cell)	1 : 100

MPO, myeloperoxidase.

Results

Healing after implant placement was uneventful at all implant sites. One male dog developed Addison's disease and was euthanized 2 months after implant placement. The clinical examination performed at the end of the plaque formation period revealed that the gingiva and the peri-implant mucosa at experimental sites were severely inflamed.

Radiological findings

Radiographs from tooth and implant sites at ligature removal and at biopsy are presented in Fig. 2. The amount of bone loss that occurred during the active breakdown period was more pronounced at both types of implants than at teeth (2.69 ± 0.57 mm for implants in group A, 3.14 ± 0.69 mm for implants in group B and 1.74 ± 0.53 mm for teeth). The difference between teeth and the two implant groups was statistically significant.

The mean bone loss that took place during the 26-week period between ligature removal and biopsy was 0.00 ± 0.53 mm for teeth, −0.02 ± 0.66 mm for implants in group A and −1.34 ± 1.19 mm for implants in group B (Table 2). The differences between implant B and implant A and between implant B and teeth, respectively, were statistically significant. Multilevel modeling revealed that neither animal nor position in mandible influenced results.

The results from the reproducibility assessments were reported previously (Albouy et al. 2012) and amounted to SD 0.04 and 0.32 mm, respectively, for the two examiners, and an inter-examiner SD of 0.24 mm.

Histological findings

The examination of the tissues sampled from the tooth sites revealed signs of established periodontitis with loss of connective tissue attachment and bone together with a distinct area of infiltrated connective tissue (ICT) in the gingival tissue (Fig. 3). A subgingival biofilm in the pocket compartment was

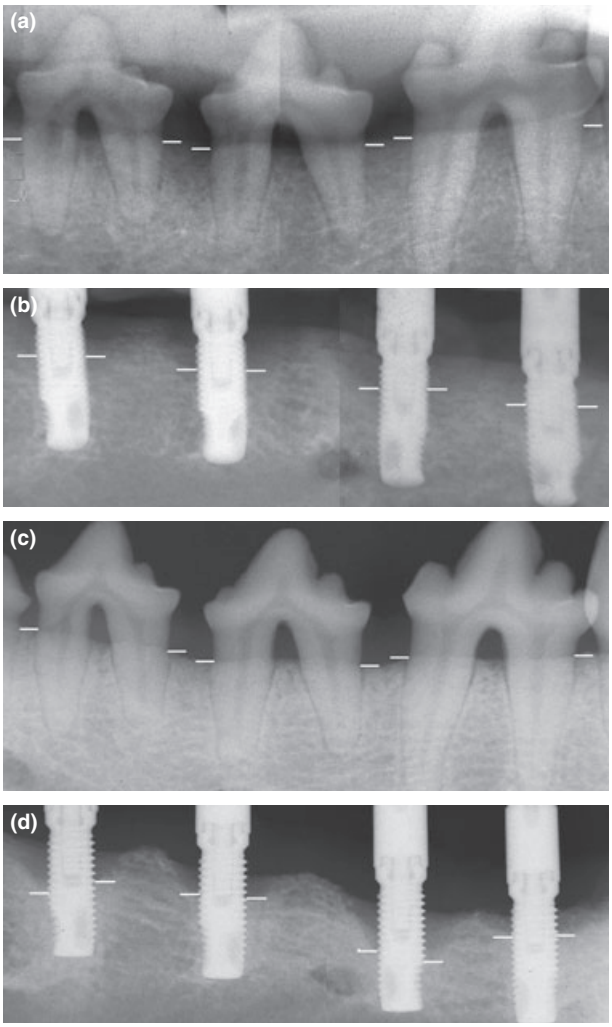

Fig. 2. Radiographs from tooth and implant sites obtained at ligature removal (baseline) (a, b) and at biopsy (26 weeks) (c, d). The arrows indicate bone levels.

Table 2. **Bone level alterations (mm) during the 6-month period following the ligature removal. Mean values and standard deviation (SD) (n = 5)**

	Tooth	Implant A	Implant B
Baseline (ligature removal) – 6 months	0.00 (0.54)*	−0.02 (0.66)†	−1.34 (1.19)*,†

*P-value <0.05 tooth vs. implant B.
†P-value <0.05 implant A vs. implant B.

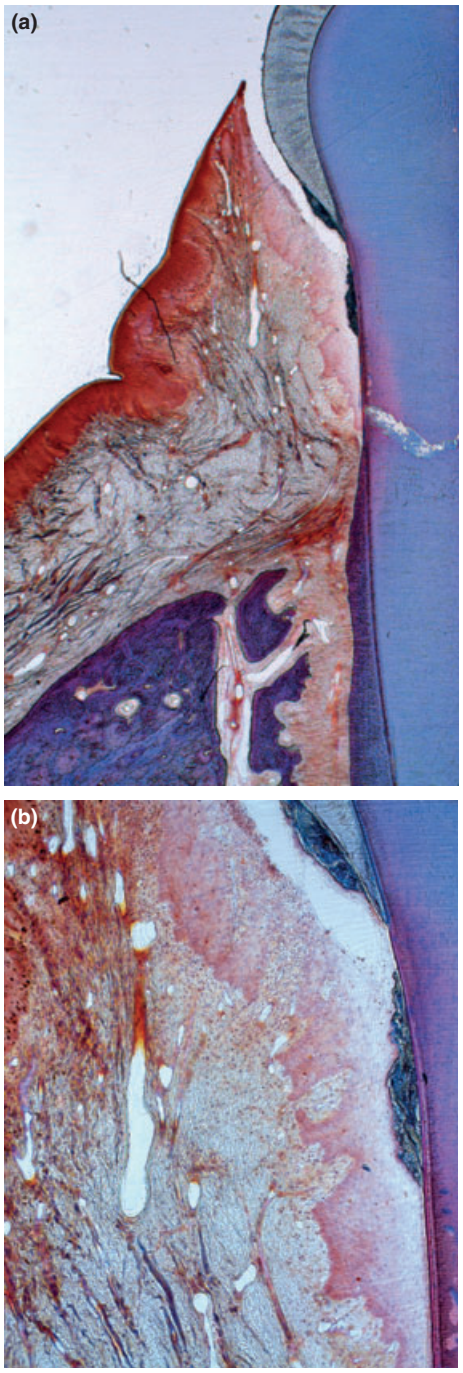

Fig. 3. Buccal-lingual ground section from a tooth site exhibiting periodontitis (a). Larger magnification from (a) illustrating pocket epithelium and infiltrated connective tissue (b). Fibrin stain of Ladewig.

separated from the connective tissue by a pocket epithelium. A zone of structurally intact and non-inflamed connective tissue was consistently present between the apical border of the ICT and the alveolar bone crest. Osteoclasts were only occasionally identified at the alveolar bone surface in the tooth sections.

The examination of the peri-implant tissues revealed a large inflammatory process in the connective tissue and an extensive osseous defect around all implants (Figs 4 and 5). An ulcerated pocket epithelium lined the inflamed part of the mucosa toward the pocket compartment, and a large area of biofilm and calculus occupied the implant surface. No epithelial barrier was present in the most apical part of the ICT and, hence, this part of the lesion was characterized as an open wound that was facing a large zone of pus. The lateral and apical portions of the ICT extended to the bone crest, the surface of which was lined with osteoclasts. Large, multi-nuclear cells were also occasionally detected in the connective tissue compartment immediately lateral to the bone crest.

Histometric measurements

The results from the histometric measurements are reported in Tables 3 and 4. Overall, vertical dimensions related to the supra-alveolar soft tissue, pocket epithelium, ICT were significantly larger at implants than at teeth. These dimensions were, in addition, also significantly larger at implants type B than at implants type A. Similar differences were also found with regard to the size of ICT and the distance between the ICT and the bone crest.

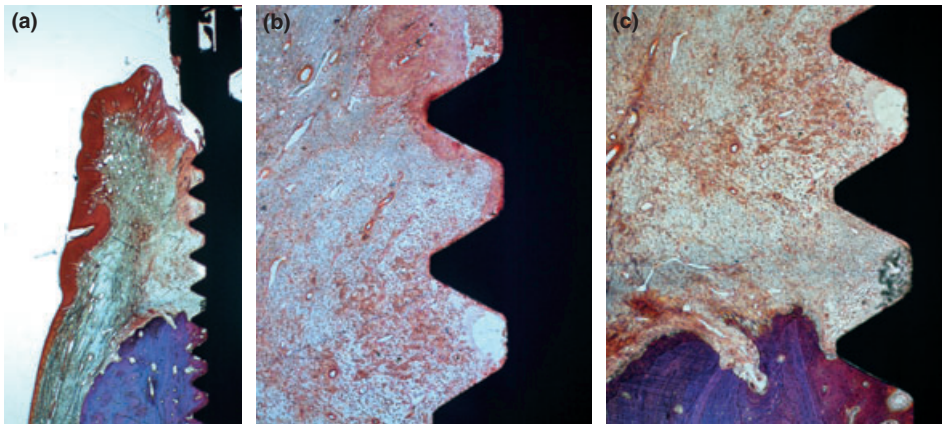

Fig. 4. Buccal-lingual ground section from an implant site representing group A exhibiting peri-implantitis (a). Larger magnification from (a) illustrating the apical part of the pocket epithelium (b) and infiltrated connective tissue and bone crest (c). Fibrin stain of Ladewig.

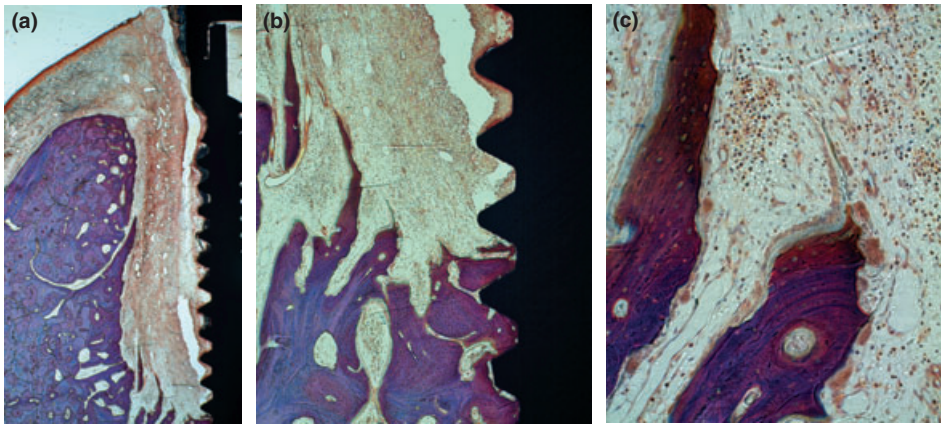

Fig. 5. Buccal-lingual ground section from an implant site representing group B exhibiting peri-implantitis (a). Larger magnification from (a) illustrating the apical part of the pocket compartment, infiltrated connective tissue and bone crest (b). Larger magnification from (b) illustrating numerous osteoclasts lining the bone crest (c). Fibrin stain of Ladewig.

Table 3. **Results from the histometric measurements at tooth and implant sites. Mean values and standard deviations (SD)**

Dimension (mm), area (mm^2)	Tooth	Implant A	Implant B
GM/PM-aPE	2.59 (0.59)*,†	3.59 (0.70)*,‡	5.01 (1.59)^{†,N}
aPlaque-aPE	0.85 (0.38)*,†	0.1 (0.69)*	−0.2 (0.58)†
aICT-B	1.32 (0.56)*,†	0.27 (0.22)*,‡	0.09 (0.15)†,‡
cICT-aICT	1.83 (0.64)*,†	4.02 (0.57)*,‡	5.25 (1.73)†,‡
ICT area	0.60 (0.36)*,†	2.43 (1.24)*,‡	3.47 (2.07)†,‡

**P*-value <0.05 tooth vs. implant A.
†*P*-value <0.05 tooth vs. implant B.
‡*P*-value <0.05 implant A vs. implant B.

A further analysis of the ICT and the bone defects found in the peri-implantitis sites revealed that not only the size of the defect area but also the vertical dimension of the intra-bony component was significantly larger at implant B than at implant A (Table 4). Similarly, the distance between the ICT and the bone crest assessed in different levels within the defect compartment was consistently smaller at implant type B than at implant type A.

Immunohistochemical features

The results from the immunohistochemical (IHC) analysis are presented in Table 5. The analysis made in the paraffin-embedded sections used for the IHC preparations once again revealed that the size of the ICT was considerably larger at both types of implants than that at teeth. The most conspicuous finding with regard to differences in the density of markers was made in relation the MPO marker (Figs 6 and 7). The proportion of such cells was 3–4 times larger at implant sites than at tooth sites and, in addition, significantly larger at implant type B than at implant type A. Analysis of morphological features of MPO-positive cells indicated the predominance of multi-nuclear cells in relation to mono-nuclear cells within this cell category. The number of TRAP-positive cells was substantially larger at peri-implantitis than at periodontitis sites. TRAP-positive cells are illustrated in Fig. 8. The difference in numbers of TRAP cells between implant type B and teeth was statistically significant.

Discussion

In the present study, the tissue reactions to plaque formation following ligature removal at teeth and implants exposed to experimental periodontitis and peri-implantitis were analyzed. It was demonstrated that the amount of bone loss that occurred during the period following ligature removal was significantly larger at implants with a modified surface than at implants with a turned surface and at teeth. The histological analysis revealed that peri-implantitis sites exhibited inflammatory cell infiltrates that were larger, extended closer to the bone crest and contained larger proportions of neutrophil granulocytes and osteoclasts than in periodontitis. It is suggested that lesions produced in experimental periodontitis and peri-implantitis are different and that implant surface characteristics influence the inflammatory process in experimental peri-implantitis and the magnitude of the resulting tissue destruction.

The present study addressed the comparison between experimental periodontitis and peri-implantitis and focused on the reaction following ligature removal in the experimental protocol. There are few experimental studies comparing periodontitis and peri-implantitis. Lindhe et al. (1992) placed cotton ligatures around teeth and implants in five beagle dogs, and plaque was allowed to accumulate. While the ligatures were removed after 6 weeks, plaque formation continued, and after an additional 4-week period, clinical and radiographic examinations were performed, and block biopsies were obtained. It was reported that clinical signs of inflammation and radiographic bone loss were more pronounced in peri-implantitis than in periodontitis sites. In addition, the histological examination revealed that the ICT was larger at implants than at teeth and that peri-implantitis lesions but not periodontitis lesions extended to the bone crest. Similar findings

Table 4. **Results from the histometric measurements related to bone defect dimensions at implant sites. Mean values and standard deviations (SD)**

Dimension (mm), area (mm^2)	Implant A	Implant B
Defect area	2.41 (1.57)*	4.06 (2.46)*
B-BC	2.03 (1.13)*	2.96 (1.41)*
BC-I	1.83 (0.74)	1.93 (0.51)
ICT-Bw coronal	0.49 (0.26)*	0.11 (0.16)*
ICT-Bw middle	0.36 (0.20)*	0.06 (0.12)*
ICT-Bw apical	0.27 (0.31)*	0.08 (0.13)*

*P-value <0.05 implant A vs. implant B.

Table 5. **Results from the analysis of immunohistochemical markers at tooth and implant sites. Mean values and standard deviations (SD)**

Area (mm^2)	Tooth	Implant A	Implant B
ICT area	0.42 (0.28) *,†	1.98 (1.54) *	2.30 (0.95)†
Cell markers	**Tooth**	**Implant A**	**Implant B**
CD3 (%)	5.39 (3,92)	5.78 (2.11)	7.08 (3.42)
CD20 (%)	4.42 (4.02)	2.61 (2.82)	1.81 (1.54)
MPO (%)	2.72 (1.49)*,†	8.53 (5.71)*,‡	13.26 (5.81)‡,†
IgG (%)	4.59 (3.15)	4.83 (2.21)	4.66 (2.91)
TRAP (n/mm) total	0.74 (1.24)†	3.62 (3.72)	6.88 (5.73)†
TRAP (n/mm) in contact with bone	0.55 (0.88)†	1.53 (1.31)	3.16 (2.51)†

*P-value <0.05 tooth vs. implant A.
†P-value <0.05 tooth vs. implant B.
‡P-value <0.05 implant A vs. implant B.
ICT, infiltrated connective tissue; MPO, myeloperoxidase; TRAP, tartrate resistant acid phosphatase.

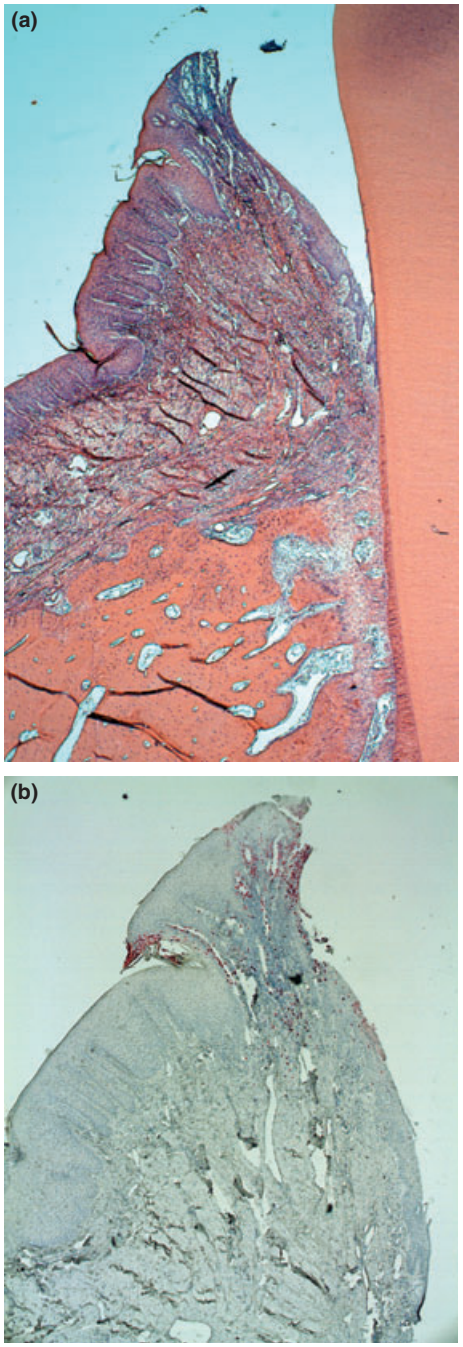

Fig. 6. Buccal-lingual, paraffin-embedded section stained in HE from a periodontitis site (a). Larger magnification from (a) with immunohistochemical-prepared myeloperoxidase marker (b). Note the location of few positive cells in the marginal portion of the gingiva.

were presented by Schou et al. (1993). They studied experimental peri-implantitis and periodontitis in monkeys and reported that bone loss was more pronounced around implants than teeth and that bone loss was associated with a high number of osteoclasts in the histological specimens. The combined radiological and histological findings presented in the studies by Lindhe et al. (1992) and Schou et al. (1993) corroborate the observations made in the current experiment and indicate that critical differences exist between peri-implantitis and periodontitis lesions.

As pointed out in a review by Berglundh et al. (2011), the ligature model is not ideal to study progression of a disease as the investigator of the experiment in several aspects controls the process. Thus, the type and the coronal-apical position of the ligature and the frequency of removing and replacing the ligature influence the amount of tissue breakdown. Comparisons between experimental periodontitis and peri-implantitis during the active breakdown period, that is, in the presence of ligatures, should therefore be made with care. The present experiment applied the new concept of removing the ligatures during the course of the experiment and analyzing tissue reactions to plaque formation in the absence of ligatures during 6 months. This new approach to the model was introduced by Zitzmann et al. (2004), who demonstrated that spontaneous progression of experimentally induced peri-implantitis could occur following the removal of ligatures. The model was subsequently applied in experiments on the influence of implant surface characteristics on spontaneous progression of experimental peri-implantitis. Berglundh et al. (2007) in study in dogs demonstrated that spontaneous progression was more pronounced around implants with rough surfaces than implants with smooth surfaces. While the implants used in the experiment by Berglundh et al. (2007) were custom made, Albouy et al. (2008, 2009) applied the modified model on commercially available implants in a study on experimental peri-implantitis in Labrador dogs. It was reported that implant surface characteristics influenced spontaneous progression of the disease. Thus, the spontaneous progression model in experimental peri-implantitis demonstrated that tissue destruction also occurs in the absence of a ligature. Similar evidence does not exist for experimental periodontitis.

The current study applied the spontaneous progression model in experimental periodontitis and evaluated outcomes in relation to experimental peri-implantitis around implants with different surface characteristics. The finding that no further bone loss was detected after ligature removal around teeth and that the lesion in the periodontitis sites was consistently separated from the alveolar bone by a zone of non-infiltrated connective tissue supports the view on the chronicity of periodontitis as an inflammatory disease. It should be made clear, however, that the biofilm formation period after ligature removal in the present study was restricted to 6 months and that longer periods of plaque exposure may result in disease progression with attachment loss and bone

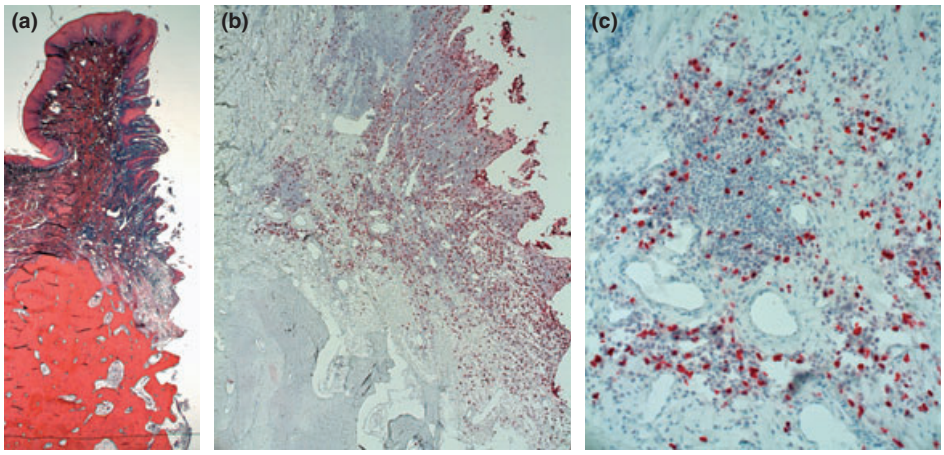

Fig. 7. Buccal-lingual, paraffin-embedded section stained in HE from a peri-implantitis site (a). Larger magnification from (a) with immunohistochemical-prepared myeloperoxidase (MPO) marker (b). Note the large number of positive cells in the profound portion of the infiltrated connective tissue. Detail of (b) indicating MPO-positive cells (c).

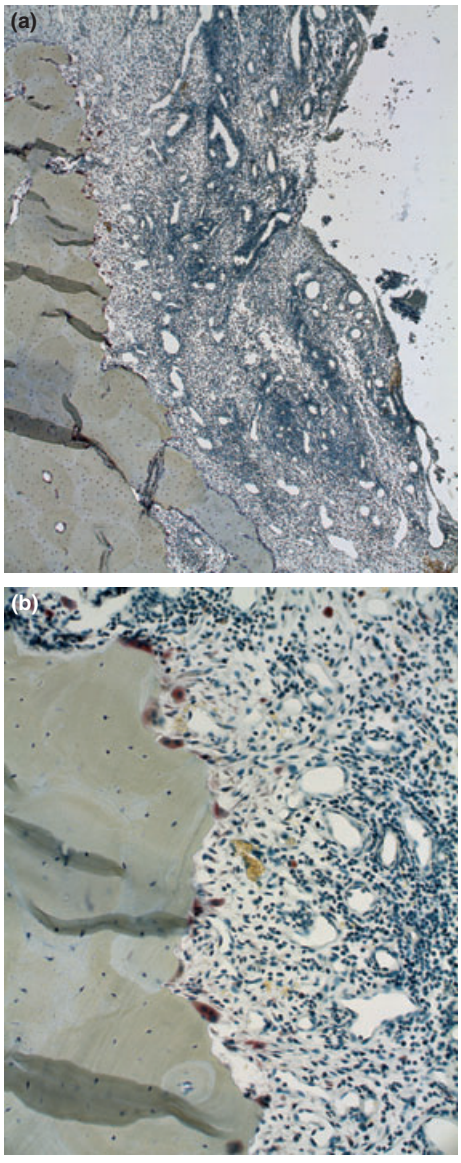

Fig. 8. Buccal-lingual, paraffin-embedded section from a peri-implantitis site (Fig. 7) illustrating tartrate resistant acid phosphatase (TRAP)-positive cells (a). Detail of (a) indicating multinucleated TRAP-positive cells on the bone crest (b).

loss. Lindhe & Ericsson (1978) evaluated the effect of the ligature model in a study on experimental periodontitis in dogs. Following an initial period of ligature-induced breakdown of periodontal tissues, ligatures were removed from some sites and kept in other sites during a subsequent 6-month period of plaque formation. While it was reported that the removal of ligatures converted an "active" progressive lesion to a "resting" lesion, no longitudinal assessments of radiographic bone loss were performed. The observation on differences between the two groups of sites in the study by Lindhe & Ericsson (1978) was based on end-point histological assessments on loss of connective tissue attachment.

The main purpose of the present study was to analyze differences between experimental periodontitis and peri-implantitis. The additional observation, however, that differences in disease progression of peri-implantitis occurred between the two types of implants used must also be emphasized. Although this particular finding has been addressed in detail elsewhere (Albouy et al. 2012), the histopathological analysis of the present experiment revealed that differences between periodontitis lesions and peri-implantitis lesions around implant A were larger than corresponding differences between implant A and implant B. The most conspicuous difference between the two diseases was the size of the ICT and the distance between the ICT and the bone crest. Thus, the ICT in peri-implantitis sites was about 4–6 times larger than that in periodontitis sites, while a reverse relationship was found regarding the distance between lesion and the bone. This observation indicates that periodontitis lesions not only occupy a smaller volume of the adjacent connective tissue than peri-implantitis lesions, but also that periodontal tissues, in contrast to peri-implant tissues, possess the ability to encapsulate the lesion and thereby separate it from the bone crest. This finding is in agreement with data presented by Lindhe et al. (1992) who described the formation of the connective tissue capsule as a "self-limiting process," which was unique for periodontal tissues. In addition, the data presented in the study by Lindhe & Ericsson (1978) on experimental periodontitis in dogs indicated that the removal of ligatures resulted in an increase in the distance between the ICT and the bone and thereby converted the site to a resting lesion.

The present study included longitudinal assessments of bone level changes in radiographs and end-point evaluations in histological sections. The preparation of histological specimens was carried out in two different ways to provide quantitative analysis of dimensions in un-decalcified ground sections and qualitative evaluations at the cellular level of lesions in paraffin-embedded decalcified sections. Cells were identified in the paraffin sections using immunohistochemical and enzyme-based techniques. Although the relative proportions of CD3−, CD20−, and IgG-positive cells did not differ between the lesions, it must be kept in mind that the size of the ICT was substantially larger in peri-implantitis than in periodontitis sites. As the density of cell types was the target for the analysis, the true number of these cells may be higher in peri-implantitis lesions. Considering the difference in size of the ICT, the large difference between periodontitis lesions and the two different groups of peri-implantitis lesions that was detected in relation to the density of MPO-positive cells needs to be acknowledged. The MPO marker in the present material indicated mainly neutrophil granulocytes, and it is apparent that periodontitis lesions contain small numbers, while peri-implantitis lesions exhibit large quantities of this cell category. In addition, the magnitude of the difference in the proportion of MPO-positive cells between the lesions around implant A and implant B indicates an association between disease progression and this particular cell group. A similar association is also evident for osteoclasts, which were identified by the TRAP-marker.

Taken together, the histological findings of the largest ICT and highest proportions of neutrophil granulocytes and osteoclasts in peri-implantitis sites around implants type B coincide with disease progression as assessed by bone loss in radiographs. In previous studies on experimental periodontitis and peri-implantitis, the cellular composition of the lesions was identified using morphological features. Lindhe et al. (1992) reported that the percentages of neutrophils and plasma cells were larger and that the proportions of lymphocytes and macrophages were smaller in peri-implantitis than in periodontitis lesions. It was also reported that osteoclasts occurred in high numbers on the bone surface facing the ICT in peri-implantitis lesions, while no osteoclasts were found in any specimen representing periodontitis lesions. The findings regarding larger densities of neutrophils and plasma cells and the large number of osteoclasts in peri-implantitis lesions reported by Lindhe et al. (1992) are in agreement with data presented in the present study. Schou et al. (1993) in a study in monkeys reported that no differences were found in densities of neutrophils and plasma cells in experimental periodontitis and peri-implantitis. Osteoclasts, however, were found in sites that exhibited bone loss.

The finding of large numbers of neutrophils in peri-implantitis lesions in the present experiment is also supported by data reported from analysis of human biopsy material. Berglundh et al. (2004) in a study on soft tissue biopsies from 12 implant sites with peri-implantitis reported that the large lesions contained not only plasma cells and lymphocytes but also PMN cells in high numbers, which were residing in peri-vascular compartments distant from the "pocket area". Gualini & Berglundh (2003) used IHC to detect inflammatory cells in mucositis and peri-implantitis lesions in humans. It was reported that B-cells and neutrophils occurred in higher numbers in peri-implantitis than in mucositis lesions. Bullon et al. (2004), however, who evaluated the composition of aggressive periodontitis and peri-implantitis lesions in humans, presented data that did not corroborate the previously reported results from human and experimental studies. Thus, Bullon et al. (2004) reported that both types of lesions contained T and B cells, plasma cells, and macrophages and that T cells occurred in larger numbers than B cells.

In summary, the present study demonstrated that lesions produced in experimental periodontitis and peri-implantitis are different with regard to size, composition, and encapsulation from bone. While the advantage in producing quantitative analysis of experimentally induced disease in animal models is obvious, qualitative evaluations of cells involved in peri-implantitis and periodontitis lesion require well-designed assessments in humans to further elucidate differences between the two diseases.

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RESEARCH REPORTS
Clinical

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Composition of Human Peri-implantitis and Periodontitis Lesions

ABSTRACT

The aim of the present study was to examine differences in cellular characteristics of human peri-implantitis and periodontitis lesions. Two groups of patients were included: 40 patients with generalized severe chronic periodontitis and 40 patients presenting with severe peri-implantitis. Soft tissue biopsies were obtained from diseased sites (probing pocket depth ≥ 7 mm with bleeding on probing) and prepared for histologic and immunohistochemical analysis. In contrast to periodontitis samples, peri-implantitis lesions were more than twice as large and contained significantly larger area proportions, numbers, and densities of CD138-, CD68-, and MPO-positive cells than periodontitis lesions. Peri-implantitis lesions also extended to a position that was apical of the pocket epithelium and not surrounded by noninfiltrated connective tissue. They further presented with significantly larger densities of vascular structures in the connective tissue area lateral to the infiltrated connective tissue than within the infiltrate. This study suggests that peri-implantitis and periodontitis lesions exhibit critical histopathologic differences, which contribute to the understanding of dissimilarities in onset and progression between the 2 diseases.

KEY WORDS: biopsy, dental implant, immunohistochemistry, inflammation, inflammatory cell, peri-implant disease.

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INTRODUCTION

Peri-implantitis is an increasing problem in implant dentistry (Mombelli *et al.*, 2012). It is recognized by bleeding on probing with loss of supporting tissues (Lindhe *et al.*, 2008; Lang *et al.*, 2011). Although clinical and radiologic signs of periodontitis and peri-implantitis have many features in common, results from experimental studies indicate that significant histopathologic characteristics exist that may explain differences in disease onset and progression (Lindhe *et al.*, 1992; Schou *et al.*, 1993; Berglundh *et al.*, 2011; Carcuac *et al.*, 2013). In a review on periodontitis and peri-implantitis lesions, Berglundh *et al.* (2011) reported that there is comprehensive information on human periodontitis lesions, while few studies have examined peri-implantitis lesions prepared from human samples. Furthermore, analysis of human peri-implantitis was made on a small number of samples and patients, and comparisons to periodontitis were exceptional.

Animal models in this field provide access to the entire disease process, including soft and hard tissues. In an experimental study of dogs, Carcuac *et al.* (2013) reported that peri-implantitis lesions were considerably larger, extended closer to the crestal bone, and contained larger number of osteoclasts than periodontitis lesions. As the findings in experimental studies need to be validated in human protocols and a more comprehensive analysis of cellular and functional characteristics of the lesions is required, evaluations of human disease samples obtained from patient groups of sufficient size and with well-described clinical characteristics of diseased sites are needed. The aim of the present study was to perform the requested assessments of human peri-implantitis and periodontitis lesions.

MATERIAL & METHODS

Two groups of patients from the Clinic of Periodontics, Mölndal, Public Dental Health Services, Västra Götaland, Sweden, were included. One group consisted of 40 patients with generalized severe chronic periodontitis (24 women and 16 men; age range, 40-89 yr; mean, 64 ± 11.45 yr). The patients exhibited bone loss ≥ 50% and probing pocket depth ≥ 7 mm with bleeding on probing at ≥ 4 teeth. A second group of 40 patients presenting with severe peri-implantitis was also recruited (23 women and 16 men; age range, 46-93 yr; mean, 70 ± 10.41 yr; function time for implants, 2-10 yr). The subjects in this group demonstrated ≥ 1 implant with peri-implant bone loss ≥ 3 mm and a peri-implant probing pocket depth ≥ 7 mm, with bleeding on probing and/or suppuration.

The study protocol was approved by the local human review board, and before enrollment, the patients of the 2 groups received information regarding the purpose of the study and signed an informed consent. None of the subjects had a known systemic disorder that could have affected the periodontal and peri-implant tissue conditions. Smoking habits were recorded in both groups.

No patients had received any treatment regarding periodontal or peri-implant diseases during the last 6 mo. On an individual basis, the patients were given a detailed case presentation and oral hygiene instruction. They also received professional supragingival tooth/implant cleaning.

Biopsy and Histologic Processing

Diseased interproximal tooth/implant sites were identified that exhibited probing pocket depth ≥ 7 mm with bleeding on probing. Following local anesthesia (Xylocain Dental Adrenalin, 20 mg/mL + 12.5 μg/mL; Dentsply Pharmaceutical, York, PA, USA), 2 parallel incisions, 4 mm apart, were made with a 12D scalpel blade (Hu-Friedy, Chicago, IL, USA) through the soft tissue until bone contact was achieved. The 2 incisions were connected with a perpendicular incision placed at a distance of 4 mm from the tooth/implant. The biopsies, including the entire supracrestal soft tissue portion of the diseased site, were carefully retrieved and prepared for histologic and immunohistochemical analysis.

The tissue samples were rinsed in saline, mounted in mesh basquets (Tissue-Tek Paraform Sectionable Cassette System; Sakura Finetek Europe, Netherlands), and placed in 4% buffered formalin for 48 hr. The samples were stored in 70% ethanol, kept at 4°C, and subsequently dehydrated and embedded in paraffin. Microtome serial sections (5 μm thick) were cut and mounted on glass poly-D-lysine-coated slides and stained with hematoxylin and eosin.

Immunohistochemistry

Immunohistochemical preparation was performed with an EnVision kit (EnVision System-HRP; DAB, DakoCytomation, Glostrup, Denmark). The primary mouse monoclonal antibody to CD3 (1:50 dilution) was used to identify T cells, while B cells, plasma cells, macrophages, and endothelial cells were detected through mouse monoclonal antibodies to CD20 (1:400), CD138 (1:50), CD68 (1:200), and CD34 (1:100), respectively. Polyclonal rabbit anti-human myeloperoxidase was used to detect polymorphonuclear leukocytes (1:1,500). The sections were dewaxed and incubated in antigen retrieval solution (DIVA; Biocare Medical, Concord, CA, USA) at 60°C over night and subsequently incubated with primary antibodies for 30 min and with Dako's Peroxidase Block for 10 min. The specimens were then incubated with a characterized and diluted mouse or rabbit primary antibody, followed by a labeled polymer for 30 min and a substrate/chromogen for 10 min. Counterstaining was performed with hematoxylin. Finally, the sections were mounted and coverslipped. Human oral mucosa tissue sections were used as positive controls, while negative controls were produced by substituting the primary antibody with nonimmune serum.

Histologic Analysis

The histologic examinations were performed in a Leica DM-RBE microscope (Leica, Heidelberg, Germany) equipped with an image system (Q-500 MC; Leica, Wetzlar, Germany). The surface area of the infiltrated connective tissue (ICT) in the connective tissue (area ICT) was evaluated by outlining its circumference with a mouse cursor.

The histologic quantitative assessments of cell markers were performed with a microscope equipped with an image system (Leitz DM-RBE Q-500 MC; Leica, Wetzlar, Germany). For the identification of positive cell markers, an interference contrast setting at a magnification of × 400 was applied as previously described (Liljenberg *et al.*, 1994; Zitzmann *et al.*, 2001). A point-counting procedure was used to determine the percentage of positive cell markers within the ICT. A lattice comprising 400 points was superimposed over the tissue area. Cross points that indicated the positive cell in the compartment to be examined were counted and related to the total counts for the entire ICT (%) and expressed as area proportions (%) of ICT. In addition, the mean size of positive cells was assessed by using a mouse cursor in 10 randomly selected sections of each category of markers in both patient groups. Based on the data on cell density and size of ICT with the cell size, the number of total positive cells for each marker in the ICT was estimated. The density of vascular structures of the ICT was determined *via* the point-counting procedure with the reference of endothelial structures expressing CD34. The density of vascular units was also performed in a 200-μm-wide zone of the connective tissue immediately lateral to the ICT. To assess the intra-individual variation of the immunohistochemical analysis, double assessments were performed within 2-mo intervals on 10 sections representing each maker used.

Data Analysis

Mean values and standard deviations were calculated for each variable and patient. Differences between patient groups were analyzed with the Student's *t* test for unpaired observations (n = 80). The null hypothesis was rejected at $p < .05$. For superiority of peri-implantitis lesions in relation to periodontitis lesions, with an α of 0.05, a given standard deviation of 1.1% to 2.5%, and a power of 80%, a difference in area proportions of cells of 3% required a sample size of 30 subjects in each group. Analysis of covariance was performed to analyze possible effects of sex, age, and smoking on the results.

RESULTS

There were no statistically significant differences regarding distribution of age and sex between the 2 patient groups. The proportion of smokers was 27.5% in both groups. Micrographs illustrating periodontitis and peri-implantitis lesions are presented in Figure 1. In the sections representing the periodontitis group, the lesion resided in a well-defined compartment of the connective tissue that was walled off by a pocket epithelium toward the pocket and a non-ICT portion on its lateral and apical aspects. In the peri-implantitis specimens, however, the ICT occupied a considerably larger portion of the connective tissue adjacent to an ulcerated pocket epithelium. In addition, the ICT in this group of specimens extended to a position that was apical of the pocket epithelium and not surrounded by a zone of non infiltrated connective tissue.

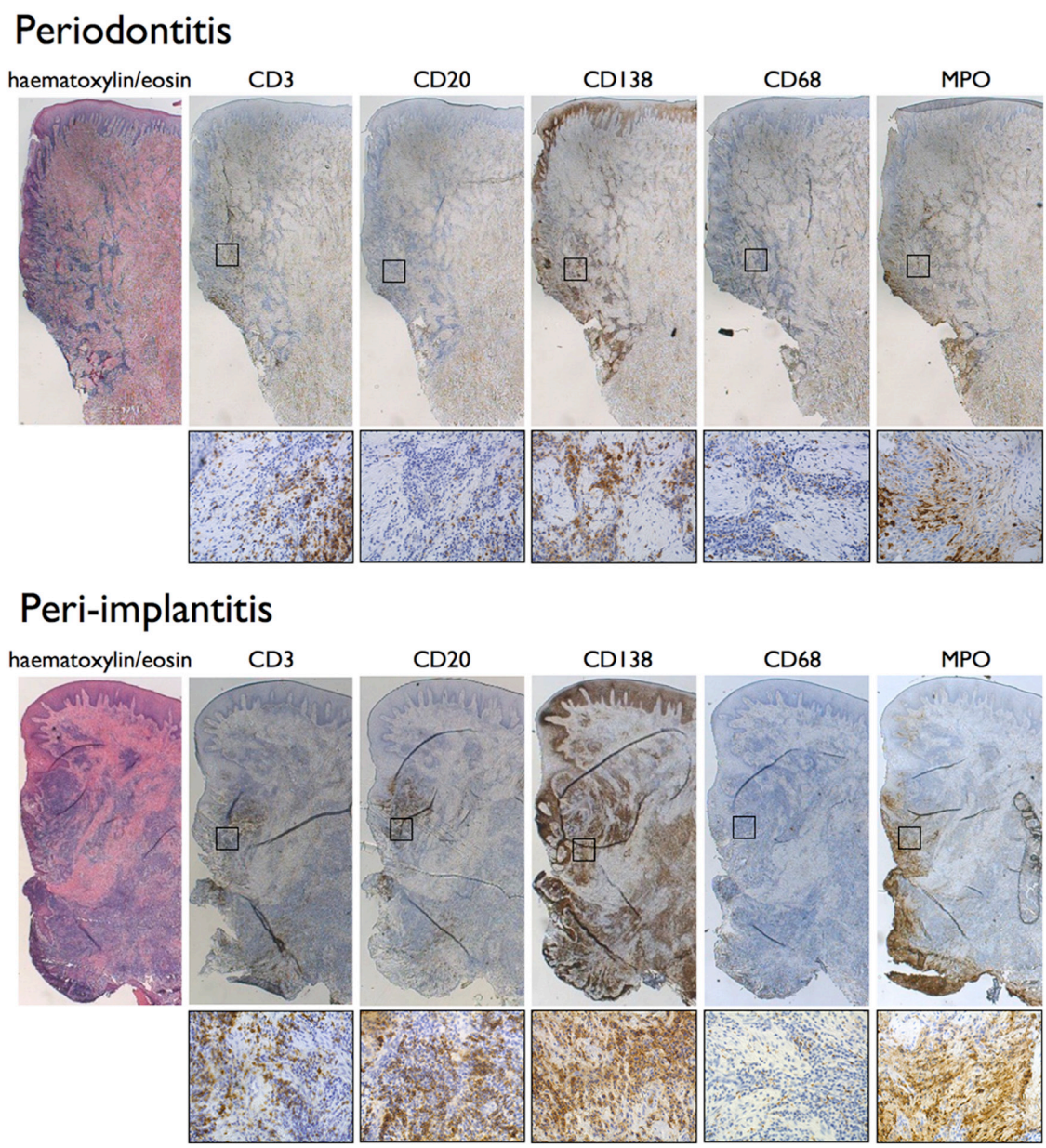

Figure 1. Sections prepared from periodontitis and peri-implantitis sites. Pocket area located to the left. Haematoxylin and eosin, CD3, CD20, CD138, CD68, and MPO markers. Magnification x25 and x400.

The results from the analysis of the size of ICT and area proportions of cell markers are reported in Table 1. The ICT in the peri-implantitis sites was more than 2 times larger than the lesions in the periodontitis sections (3.48 ± 2.54 mm^2 *vs.* 1.49 ± 1.05 mm^2). This difference was statistically significant. The area proportions of the ICT that was occupied by CD138-, CD68-, and MPO-positive cells were significantly larger in peri-implantitis than in periodontitis specimens, while a reverse relationship was found for CD20-positive cells. The density of vessels within the ICT was significantly larger in periodontitis than in peri-implantitis. In the connective tissue portion lateral to the ICT, however, the proportion of vascular structures was significantly larger in peri-implantitis than in periodontitis. In addition, the differences in vascular density between the 2 tissue compartments were statistically significant for both periodontitis and peri-implantitis specimens.

The percentage distribution of total number of cells in ICT of periodontitis and peri-implantitis lesions with the relative overall size of the ICT is depicted in Figure 2. This figure also illustrates the large discrepancy on the overall size of the ICT between the 2 types of specimens.

The results from the assessments of cell size, the calculated total number positive cells, and number of cells/mm^2 within the ICT are reported in Table 2. The estimated total number of inflammatory cells within ICT was significantly larger in peri-implantitis than in periodontitis sections. The numbers of CD3-, CD138-, CD68-, and MPO-positive cells were significantly larger in peri-implantitis than in periodontitis lesions. The

Table 1. Size and Area Proportions of ICT for Positive Cells and Vascular Units of Periodontitis and Peri-implantitis Sites

	Periodontitis (n = 40)	Peri-implantitis (n = 40)
Size of ICT (mm^2)	1.49 ± 1.05	3.48 ± 2.54*
% area proportions of ICT		
CD3	7.82 ± 5.36	6.87 ± 4.42
CD20	4.97 ± 5.23*	3.10 ± 2.79
CD138	8.96 ± 6.71	13.24 ± 9.22*
CD68	2.13 ± 3.17	3.68 ± 3.53*
MPO	4.28 ± 2.52	10.90 ± 7.53*
Vascular units within the ICT	7.81 ± 5.09*	2.75 ± 2.60
Vascular units lateral to the ICT	2.31 ± 2.34	8.58 ± 8.93*

Values in mean ± SD.
ICT, infiltrated connective tissue.
*$p < .05$.

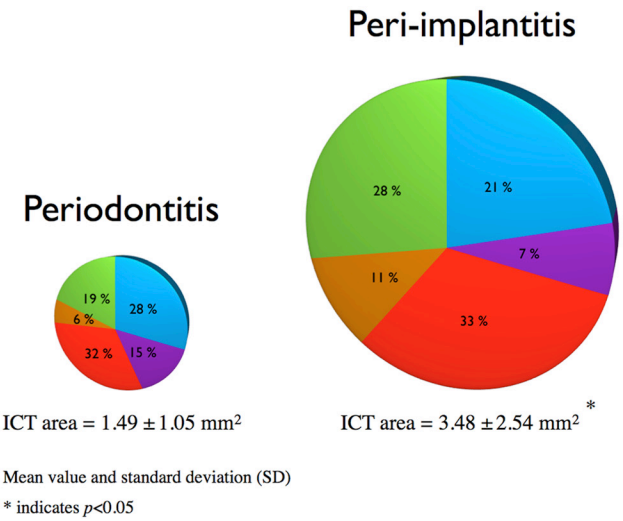

Figure 2. Percentage distribution of total number of cells in periodontitis and peri-implantitis lesions. CD3 (blue), CD20 (purple), CD138 (red), CD68 (brown), and MPO (green). Note the difference in size of infiltrated connective tissue (ICT). n = 80.

overall density of inflammatory cells within the ICT (*i.e.*, the number of cells/mm^2) was significantly higher in peri-implantitis than in periodontitis specimens. Specifically, the densities of CD138-, CD68-, and MPO-positive cells were significantly higher in peri-implantitis than in periodontitis lesions, whereas an opposite association was observed for CD20-positive cells. The largest total number of cells or cells/mm^2 among the different phenotypes was found for MPO- and CD138-positive cells in peri-implantitis lesions. These 2 cell categories in peri-implantitis not only occurred in 3- to 6-times larger numbers than their counterparts in periodontitis lesions but also outnumbered other cell groups in both types of lesions.

The analysis of covariance of patient characteristics revealed that distributions of sex, age, and smokers between the periodontitis and the peri-implantitis groups did not influence the results from the histologic assessment.

DISCUSSION

This study evaluated histopathologic characteristics in human periodontitis and peri-implantitis lesions. It demonstrated that peri-implantitis lesions were more than twice as large and contained significantly larger area proportions, numbers, and densities of CD138-, CD68-, and MPO-positive cells than periodontitis lesions. Peri-implantitis specimens, in contrast to periodontitis samples, also presented with significantly larger densities of vascular structures in the connective tissue area lateral to the ICT than within the infiltrate. The study suggests that peri-implantitis and periodontitis lesions exhibit critical histopathologic differences, which contribute to the understanding of dissimilarities in onset and progression between the 2 diseases.

As previous reports on evaluations of differences between human peri-implantitis and periodontitis lesions are few and included small numbers patients, the present study aimed at performing a comprehensive examination of histopathologic differences between the 2 diseases. Thus, the number of patients in each group (n = 40) and the severity of the conditions in the selected sites and cases suffice necessary requirements of statistical power and distinctions of clinical signs of disease. In addition, sampling of biopsies in both diseases was, in most cases, carried out in conjunction with surgical therapy. From an ethical point of view, sampling of biopsies under such conditions is restricted to the soft tissue component, as the supporting bone is not accessible. Although the biopsy-sampling procedure is aimed at including the entire supracrestal soft tissue portion of the diseased site, small parts of the apical portions of the lesion may occasionally, for technical reasons, not be retrievable in narrow osseous defects. Yet, biopsies obtained from animal experiments include the entire peri-implant and periodontal hard and soft tissue components and may, from such a perspective, be superior to the human protocol. Indeed, in an experimental study from our laboratory, Carcuac *et al.* (2013) reported that experimentally induced peri-implantitis lesions were larger and extended closer to the bone crest than periodontitis lesions. The finding on differences in size of the lesions between the 2 conditions reported by Carcuac *et al.* corroborates data presented in the present study.

Table 2. Cell Size, Total Estimated Number, and Density of Positive Cells in the ICT of Periodontitis (n = 40) and Peri-implantitis Sites (n = 40)

	CD3	CD20	CD138	CD68	MPO
Cell size (μm^2)	58 ± 4.08	63 ± 0.62	61 ± 0.43	95 ± 7.8	44 ± 1.02
Total no. of cells in ICT					
Periodontitis	2,138 ± 2,015	1,235 ± 1,683	2,624 ± 2,898	280 ± 375	1,492 ± 1,310
Peri-implantitis	4,672 ± 5,340*	1,817 ± 2,129	9,140 ± 10,850*	1,364 ± 2,016*	10,035 ± 12,366*
No. of cells per mm^2					
Periodontitis	1,348 ± 924	788 ± 829*	1,464 ± 1,096	206 ± 324	983 ± 579
Peri-implantitis	1,185 ± 762	464 ± 437	2,164 ± 1,506*	388 ± 372*	2,505 ± 1,730*

Values in mean ± SD.
ICT, infiltrated connective tissue.
*$p < .05$.

While some descriptive studies on peri-implantis lesions were presented previously, reports on comparisons between human peri-implantitis and periodontitis lesions are scarce. Sanz *et al.* (1991) analyzed soft tissue biopsies from 6 patients with peri-implantitis and reported that about two-thirds of the connective tissue portion of the biopsy was occupied by an infiltrate consisting of plasma cells, mononuclear cells, and enlarged blood vessels. Berglundh *et al.* (2004) analyzed soft tissue biopsies obtained from 12 implant sites with severe peri-implantitis in 6 patients. The histologic analysis demonstrated that the lesion occupied almost the entire connective tissue compartment and extended apical of the pocket epithelium. These observations are in agreement with results presented in the current study. In fact, the data on the mean size of 3.61 mm^2 of the ICT presented in the study by Berglundh *et al.* (2004) are consistent with results in the current report. Bullon *et al.* (2004) analyzed soft tissue biopsies from 5 cases with peri-implantitis and 5 patients with aggressive periodontitis. They reported that peri-implantitis and periodontitis lesions both presented with plasma cells, macrophages, and lymphocytes, among which T cells were more common than B cells. Similar findings were also presented by Cornelini *et al.* (2001) in a study on biopsies prepared from 10 patients with peri-implantitis.

The 2 lesions examined in the present study did not only differ in regard to their size, as the numbers and densities of CD138- (plasma cells), CD68- (macrophages), and MPO-positive cells (PMN cells) were larger in peri-implantitis than in periodontitis lesions. These differences indicate that the inflammatory response in peri-implantitis sites is more intense by promoting cells, which are part of both the innate and the adaptive host response. Studies on gene expression of proinflammatory markers in periodontitis and peri-implantitis sites have presented similar findings. Venza *et al.* (2010) analyzed soft tissue biopsies collected from different patient groups and reported that peri-implantitis sites exhibited higher mRNA expression of IL-6, IL-8, and TNFα than periodontitis. In a study on genome-wide transcriptome profiles in gingival specimens obtained from small patient groups with periodontitis and peri-implantitis, Becker *et al.* (2012) concluded that the 2 conditions represent distinct entities with different mRNA signatures.

The examination of the 2 types of lesions in the present study is relevant in regard to similar appraisals of differences between peri-implant mucositis and peri-implantitis lesions presented by Gualini and Berglundh (2003). They examined immunohistochemical characteristics of soft tissue biopsies obtained from 16 patients and reported that peri-implantitis lesions contained significantly greater proportions of B cells and elastase-positive cells (indicating PMN cells) than mucositis lesions. Thus, the severity of a condition appears to correlate with the size of the lesion and with a cell profile based on enhanced densities and numbers of the B-cell or plasma cell line with neutrophil granulocytes and macrophages. Peri-implantitis lesions carry such characteristics.

In the study on experimental peri-implantitis and periodontitis referred to earlier, Carcuac *et al.* (2013) reported that periodontitis lesions, in contrast to peri-implantitis lesions, were consistently walled off from the alveolar bone by a zone of non infiltrated connective tissue and that the biofilm in the pocket was separated from the connective tissue by a pocket epithelium. These structural differences appear to be the fundament to the dissimilar histopathologic characteristics of the 2 conditions and explain the findings in the present study on larger numbers and densities of plasma cells and neutrophils in peri-implantitis lesions.

Another observation in the current investigation was the difference in vascular density between the 2 types of lesions. As the healthy supracrestal connective tissue portion around teeth contains larger amounts of vascular structures than does the corresponding tissue compartment around implants (Berglundh *et al.*, 2004), it is likely that an inflammatory infiltrate occupying this zone would exhibit a similar difference in vascular units. Yet, data on vascular densities in peri-implant and periodontal tissues are conflicting. Bullon *et al.* (2004) used the endothelial marker CD34 and reported that the connective tissue lateral to the junctional/sulcular epithelium in peri-implantitis sites contained a larger vascular density than that in periodontitis sites. The restriction of analysis to the coronal part of the tissue in the study by Bullon *et al.* may explain the difference in results on vascular density to the present study.

It should also be noted that the connective tissue zone lateral to peri-implantitis lesions in the present material presented with enhanced density of vessels. This finding indicates a longer distance from blood vessels to target sites for transmigrating neutrophil granulocytes in peri-implantitis lesions. Taken together, the increased peripheral vascular density and the lack

of an epithelial lining between the lesion and the biofilm in the pocket may explain the dominance of neutrophil granulocytes in peri-implantitis lesions as a major difference to lesions in periodontitis.

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The effect of the local use of chlorhexidine in surgical treatment of experimental peri-implantitis in dogs

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Abstract

Aim: To evaluate the effect of surgical treatment of experimental peri-implantitis at implants with different surface characteristics using different anti-infective procedures.

Material and methods: Four implants with different surface characteristics (A: TiOblast, B: OsseoSpeed, C: AT-I, D: TiUnite) were installed in a randomized order in each side of the mandible in 6 labrador dogs 3 months after tooth extraction. Experimental peri-implantitis was induced 3 months later. Surgical treatment of peri-implantitis was performed. The implants were cleaned with gauze soaked in either saline (control) or chlorhexidine (test). Clinical and radiographical examinations were performed and microbiological samples were taken during a 6-month period after surgery. Biopsies were obtained and prepared for histological analysis.

Results: Clinical signs of soft tissue inflammation were reduced after surgical therapy in most test and control sites. While the analysis of bone level alterations in radiographs together with histological and microbiological assessments of resolution of peri-implantitis lesions failed to demonstrate statistically significant differences between test and control procedures, the evaluations disclosed significant differences between implant D and implants A, B and C on treatment outcome.

Conclusion: It is suggested that (i) the local use of chlorhexidine has minor influence on treatment outcome, (ii) resolution of peri-implantitis following surgical treatment without the adjunctive use of local and systemic antimicrobial agents is possible and (iii) the results are influenced by implant surface characteristics.

Key words: animal experiment; antiseptics; biopsy; bone; dental implant; histology; implant surface; inflammation; lesion; peri-implant disease; radiology

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Conflict of interest and source of funding statement

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Peri-implantitis is by definition an infectious disease and treatment should therefore include anti-infective procedures (Lindhe & Meyle 2008). The evaluation of different treatment protocols has called for proper experimental models that mimic natural disease and provide sufficient tools for evaluation of treatment outcomes. Experimental disease models in peri-implantitis have included procedures that frequently were used in experimental periodontitis (Berglundh et al. 2011). Thus, the combination of plaque formation and placement of ligatures around teeth or implants resulted in the establishment of lesions in gingival or peri-implant connective tissue and loss of supporting tissues (Lindhe et al. 1992, Lang et al. 1993, Schou et al. 1993). In addition, bone defects produced in experimental peri-implantitis presented with morphology similar to

that occurring in patients with peri-implantitis (Schwarz et al. 2007).

Models of experimental peri-implantitis are fundamental for the research on treatment of the disease. In a review on quality of reporting on pre-clinical research on peri-implant disease, ligature-induced peri-implantitis in canines was the most commonly used model in the research on treatment procedures (Schwarz et al. 2012). Such experiments provided results from clinical, radiological and histological evaluations to assess resolution of peri-implantitis lesions. While treatment protocols often included surgical access to implants presenting with peri-implantitis, numerous protocols including chemical agents, air-abrasives or lasers, have been presented to achieve decontamination of implant surfaces. Claffey et al. (2008) in a review on surgical treatment of peri-implantitis concluded that open debridement including implant surface decontamination procedures resolved peri-implantitis lesions and promoted bone fill. No single decontamination method, however, was found to be superior. The fact that decontamination procedures can promote resolution of peri-implantitis lesions was in part supported by results from experimental studies on osseointegration presented by Kolonidis et al. (2003), Alhag et al. (2008) and Mohamed et al. (2010) They removed implants that had been exposed to biofilm formation and, following implant surface decontamination, installed the implants in new recipient sites. Osseointegration occurred at previously contaminated parts of the implants. Resolution of peri-implantitis lesions following decontamination of implant surfaces was also reported by Persson et al. (2001) and Parlar et al. (2009).

While implant surface decontamination procedures in previous experiments on treatment of peri-implantitis often included the use of gauze soaked in chlorhexidine or saline, the effect on resolution of peri-implantitis lesions was rarely addressed. In addition, few studies evaluated the influence of implant surface characteristics on treatment outcomes (Wetzel et al. 1999, Albouy et al. 2011). The aim of this study was to evaluate the effect of surgical treatment of experimental peri-implantitis at implants with different surface characteristics using different anti-infective procedures.

Material and Methods

Animals

Six male, 19-month-old destination-bred Labrador dogs (mean weight 22 kg) were used. The study protocol was approved by the regional Ethics Committee for Animal Research, Göteborg, Sweden, approval Dnr 221-2009. The entire experiment was conducted at the Laboratory of Experimental BioMedicine at the Sahlgrenska Academy, University of Gothenburg in 2011. ARRIVE guidelines (Kilkenny et al. 2011) were followed. During all surgical procedures general anaesthesia was induced with intravenously injected Propofol (10 mg/ml, 0.6 ml/kg) and sustained with N_2O:O_2 (1:1.5-2) and Isoflurane employing endo-tracheal intubation.

Implant placement

All mandibular premolars and the first, second and third maxillary premolars were extracted. 3 months later mucoperiosteal flaps were elevated in both sides of the mandible and 4 osteotomy preparations were made in each of the premolar regions. Using a non-submerged technique, four implants with different surface characteristics were installed: implants A, B and C were 3.5 × 11 mm (Astra Tech Implant System™, Dentsply Implant IH AB, Mölndal, Sweden) and presented with a TiOblast surface (implant A), Osseospeed surface (implant B) and AT-I surface (implant C) (Johansson et al. 2012). Implant D was 3.3 × 11.5 mm with a TiUnite surface (NobelBiocare AB, Göteborg, Sweden). The sequence of implant placement was identical in both sides of each dog but randomized between animals. Healing abutments were connected to the implants and the flaps were adjusted and sutured. The sutures were removed 2 weeks later and a plaque control regimen was initiated three times a week.

Experimental peri-implantitis

Three months after implant installation experimental peri-implantitis was initiated. Thus, the oral hygiene procedures were abandoned and cotton ligatures were placed in a submarginal position around the neck portion of all implants in a manner previously described (Zitzmann et al. 2004).

A set of radiographs was obtained from all implant sites using a customized film holder (Kerr Hawe, Bioggio, Switzerland) as previously described by Persson et al. (1999) and Albouy et al. (2008, 2011). The radiographs were analysed in an Olympus SZH10 stereo macroscope (Olympus optical co, GmbH, Hamburg, Germany) and digital images were obtained with a Leica DFC280 camera (Leica, GmbH, Wetzlar, Germany). In the radiograph, the vertical distance between the abutment-implant junction and the marginal bone was assessed at the mesial and distal aspects of each implant using the QWin software (Leica Qwin Standard V3.2.0, Leica Imaging Systems Ltd., Cambridge, UK). Double assessments were made by two examiners with a 2-month interval.

The ligatures were replaced at weeks 3 and 6 and finally removed after 9 weeks. Oral hygiene procedures were re-instituted at the implants immediately after ligature removal. Microbiological samples were obtained from all experimental peri-implantitis sites 4 weeks later. Cotton rolls were used to isolate the experimental areas to avoid saliva contamination. Supragingival plaque was removed by a sterile gauze soaked in saline. Four sterile medium sized paper points (Dentsply, Maillefer, size 35, Ballaigues, Switzerland) were inserted into the most apical part of the peri-implant pocket and held in place for 10 s. Samples were taken from all implants in each animal. The paper points were removed and placed in Eppendorf tubes (Starlab, Ahrensburg, Germany) for microbiological analysis.

Microbiological analysis

The microbiological samples were analysed by the checkerboard DNA–DNA hybridization technique (Socransky et al. 1994) modified according to Papapanou et al. (1997), Dahlén (2006), Dahlén et al. (2012). The checkerboard panel included 10 dog strains and two

human strains. For details with regard to characteristics of the strains see Dahlén et al. (2012). Whole genomic DNA-probes, digoxigenin-labelled, were prepared using the High-Prime labelling kit (Boehringer-Mannheim, Germany).

Treatment of peri-implantitis

Treatment of peri-implantitis was performed at all implants 4 weeks after ligature removal. The treatment included surgical debridement of the implant sites and two different implant surface decontamination procedures, saline (control group) or 0.2% chlorhexidine (test group), one on each side of the mandible, were randomly and equally allocated in a split-mouth design. Thus, full-thickness flaps were raised on the buccal and lingual aspects of all implants and the inflamed tissue within the crater formed bone defects was removed. If present, calculus was removed from the implant surface by the use of curettes. In one side of the mandible, the implants were carefully cleaned during 3 minutes by sterile 10 × 10 mm gauze soaked in saline, whereas in the contra-lateral side cleaning of implants was performed using sterile mini-gauze soaked in 0.2% chlorhexidine. The flaps were repositioned and sutured. The sutures were removed after 2 weeks and mechanical infection control procedures were reinstituted. Clinical and radiological examinations were performed and repeated at 2, 3, 4 and 6 months after surgery. Microbiological samples were taken at 3 and 5 months of follow-up.

Biopsy and histological preparation

Six months after peri-implantitis surgery the dogs were killed with a lethal dose of Sodium-Pentothal® (Hospira Enterprises B.V., Hoofddorp, Netherlands) and perfused through the carotid arteries with a fixative (4% formaldehyde). The mandibles were retrieved and stored in the fixative. Tissue blocks containing the implant and the surrounding soft and hard tissues were dissected using a diamond saw (Exakt, Kulzer, Norderstedt, Germany) and processed for ground sectioning according to the methods described by Donath & Breuner (1982).

The tissue samples were dehydrated in increasing grades of ethanol and embedded in Technovit 7200 VLC-resin (Kulzer, Friedrichsdorf, Germany) and prepared as described previously (Carcuac et al. 2013). From each block, two parallel sections were obtained in a mesio-distal plane and two parallel sections obtained in a bucco-lingual plane. The sections were reduced by micro-grinding (Exakt, Apparatebau, Norderstedt, Germany) to a final thickness of about 30 μm and stained in toluidine blue and fibrin stain of Ladewig (Donath & Breuner 1982).

Histological analysis

The histological examinations were performed in a Leica DM-RBE microscope (Leica, Heidelberg, Germany) equipped with an image system (Q-500 MC, Leica, Wetzlar, Germany). In the ground sections, the following landmarks were identified and used for the linear measurement: the peri-implant mucosa margin (PM), the apical termination of pocket epithelium (aPE), the marginal position of bone-to-implant contact (B) and the most coronal extension of the bone crest (BC). When indicated, areas of the residual intra-bony defect (defined by the bone wall between B and BC) and of the infiltrated connective tissue (ICT) were identified and traced using a mouse cursor. Double assessments were made with a 2-month interval. The occurrence of an ICT was scored as follows:

-Score 0: no or only scattered inflammatory cells identified in an area < 1 mm^2
-Score 1: scattered inflammatory cells located in an area < 2 mm^2
-Score 2: clusters of inflammatory cells presented in infiltrates of a total area < 3 mm^2
-Score 3: abundance of inflammatory cells in a total ICT area > 3 mm^2

Data analysis

The SPSS 12.0 software package (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Mean values for all variables were calculated for each implant in each animal. Using the animal as the statistical unit ($n = 6$), differences were analysed using analysis of variance (ANOVA) and the Student-Newman–Keuls test. A p-value <0.05 was considered as significant. A statistical programme specifically designed for multilevel modelling (MLwiN 2.02; Centre for Multilevel Modelling at University of Bristol, Bristol, UK) was used to investigate the influence of dogs, sites and implant surface-related covariates on the outcome variables.

Sample size was based on intra-individual evaluations of resolution of ICT in sections and differences of 1.0 mm in radiological bone level change between groups, SD 0.3–0.7 mm, significance level of 5% and 80% power.

Results

Three months after peri-implantitis surgery, one implant B representing the test group was lost. During the period after surgical therapy clinical signs of inflammation in the peri-implant mucosae were gradually reduced and towards the end of the experiment most sites demonstrated absence of clinical signs of inflammation. At implants type D of the control group (saline), however, swelling and redness persisted in the peri-implant mucosa.

Radiological findings

Radiographs from the different implant sites at 2 weeks (baseline) after surgical therapy and at the final examination and biopsy (6 months) are presented in Fig. 1. The results from the radiological measurements are reported in Table 1 and Fig. 2.

The amount of bone loss that occurred during the preparatory period of ligature-induced breakdown varied between 3.57 ± 0.63 mm and 3.73 ± 0.47 mm. For the implant B that was lost during the follow-up period of the peri-implantitis surgery, the radiological bone loss was assessed to the apical position of the implant.

The radiological analysis failed to demonstrate statistically significant differences between test and control procedures. While implant B, C and D presented with larger mean bone loss for control than for test

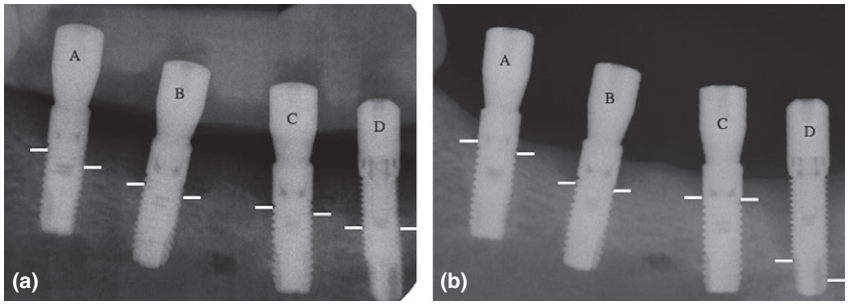

Fig. 1. Radiographs from implant A, B, C and D (from left) obtained 2 weeks after surgical treatment (baseline) (a) and at biopsy (6 months) (b). The arrows indicate bone levels.

Table 1. Radiographical bone level alterations (mm) during the preparatory period prior to treatment and after surgical treatment of peri-implantitis at test (chlorhexidine) and control (saline) sites. Mean values and standard deviation (SD) ($n = 6$)

	Implant A	Implant B	Implant C	Implant D
Preparatory period before surgical treatment	−3.58 (0.76)	−3.72 (0.65)	−3.73 (0.47)	−3.57 (0.63)
After surgical treatment				
Test group (chlorhexidine)	−0.46 (1.39)	−0.18 (2.64)	0.73 (0.81)	−1.15 (2.01)
Control group (saline)	0.37 (2.02)	−0.20 (1.88)	0.51 (1.24)	−2.77 (1.58)*

**p*-value <0.05 implant D *versus* implants A, B and C of the control group.

procedures, a reverse relationship on bone loss was assessed for implant A. The results of the analysis of the implants in the control group also revealed that bone loss at implant D was significantly larger than at implants A, B and C. Implants of type C exhibited bone gain in both control and test procedures. The results from the reproducibility assessments of the radiological measurements revealed an inter-examiner SD of 0.1 mm.

Histological findings

Ground sections produced from the different types of implants at control and test sites are presented in Fig. 3. The peri-implant mucosa around test implants of group B and C exhibited a barrier epithelium of varying length, apical of which a fibrotic connective tissue portion was observed. The majority of specimens representing implant A and D in the test group presented with inflammatory cells residing in the connective tissue compartment lateral and apical to the barrier/pocket epithelium.

In the ground sections representing A and C implants of the control group, the peri-implant mucosa exhibited a thin barrier epithelium and apical to this epithelium a non-inflamed connective tissue was facing the implant surface. Scattered inflammatory cells were occasionally found in the marginal portion of the connective tissue around the implants of group A and C. The majority of control specimens representing implant B exhibited clusters of inflammatory cells of varying size in the marginal portion of the peri-implant connective tissue. No signs of resolution of peri-implantitis were detected in control sections representing implant D. Thus, in this category of specimens an ulcerated pocket epithelium lined the inflamed portion of the connective tissue towards the pocket compartment and a large area of biofilm and calculus occupied the implant surface. Extensive osseous defects were associated with the large inflammatory cell infiltrates in the connective tissue around all implants of type D.

Histometric measurements

The results from the histometric measurements are reported in Table 2. The apical extension of the barrier/pocket epithelium (PM-aPE) varied between 1.9 and 4.6 mm, whereas the height of the supra-alveolar connective tissue (aPE-B) varied between 2.1 and 2.7 mm. The size of the residual bony defect extended from 1.5 to 8.9 mm^2. No statistically significant differences were found between test and control sites for any of the implant types. Among the control group specimens, however, the residual bony defect area at implants D was significantly larger than that of implants A, B and C.

The results of the assessments of the ICT scores are presented in Fig. 4. The overall distribution of scores differed between the test and control groups. While in implants B, C and D the test procedure resulted in lower scores than the control procedure, a reverse relationship was found for implants A. Marked differences in score distribution were also detected between the implant types. Thus, in the test group 5 of 6 implants of type C and 4 of 6 implants of type B exhibited an ICT score 0, whereas the majority of implants of type A and D presented with a score 3. In the control group the largest proportion of implants with score 0 was found among implants A, whereas 83% of implants D had an ICT score 3. The reproducibility of assessments on ICT area revealed an intra-examiner SD of 0.13 mm^2.

Microbiological analysis

The results from the microbiological analysis are reported in Table 3 and Fig. 5. In terms of total count of bacteria, no statistically significant differences were observed among implants prior to surgery. The total count, however, had decreased significantly at 3 and 5 months after surgery in both test and control groups, except for implants D. An increase in the total DNA-probe counts occurred at implant D of the control group. Statistically, significant differences in DNA-probe counts were observed between implant C and D both at 3 and 5 months. No statistically significant differences were found between test and control sites for any of the implant types.

Discussion

This study evaluated the effect of surgical treatment of experimental

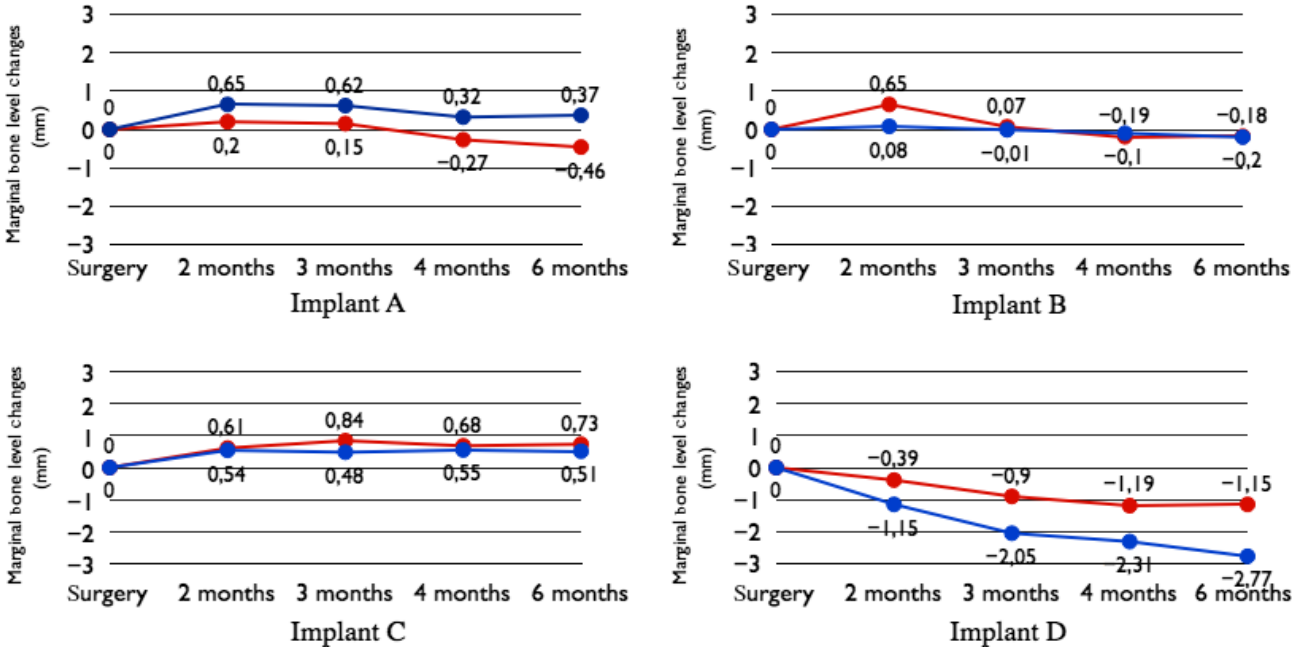

Fig. 2. Radiographical bone level changes (mm) after surgical treatment of peri-implantitis for each implant type. Mean values for test (red) and control (blue) sites. (n = 6).

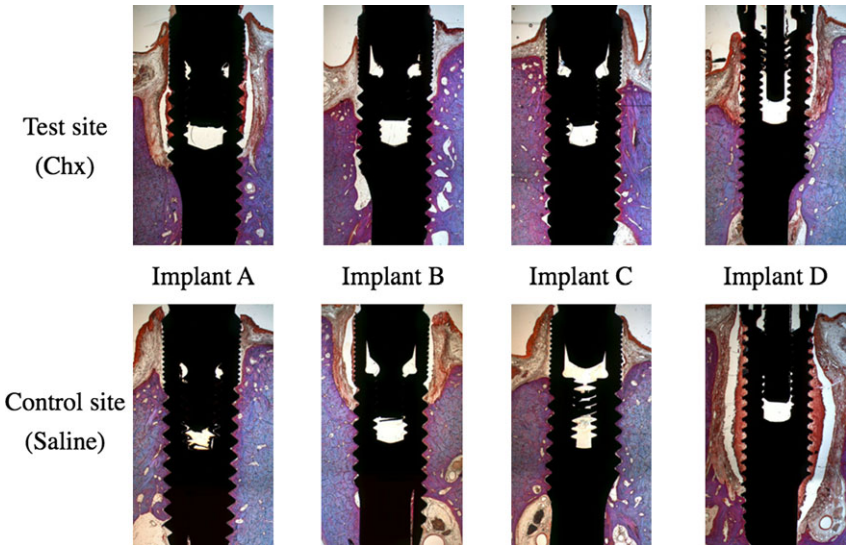

Fig. 3. Ground sections from test (chlorhexidine) and control (saline) sites representing implant types A, B, C, D.

peri-implantitis at implants with different surface characteristics using different anti-infective procedures. It was demonstrated that clinical signs of soft tissue inflammation were reduced after surgical therapy in most test (chlorhexidine) and control (saline) sites. While the analysis of bone level alterations in radiographs together with histological and microbiological assessments of resolution of peri-implantitis lesions failed to demonstrate statistically significant differences between test and control procedures, the evaluations disclosed significant differences between implant D and implants A, B and C on treatment outcome. It is suggested that (i) the local use of chlorhexidine has minor influence on treatment outcome, (ii) resolution of peri-implantitis following surgical treatment without the adjunctive use of local and systemic antimicrobial agents is possible and (iii) the results are influenced by implant surface characteristics.

Different implant surface decontamination procedures have been applied in pre-clinical in vivo experiments. Although the method of using gauze soaked in chlorhexidine or saline was commonly used in surgical treatment of experimental peri-implantitis, the two detergents were applied alone or in combination. In addition, in contrast to the target of this study, most experiments did not focus on the resolution of peri-implantitis lesions as a main outcome variable, as the degree of bone fill and potential "re-osseointegration" were also addressed. Thus, Wetzel et al. (1999) in a study in dogs, analysed treatment of experimental peri-implantitis using 0.12% chlorhexidine to decontaminate implant surfaces. It was reported that bone fill occurred in the osseous defects around all types of implants following therapy. Similar results were reported in a study performed in dogs by You et al. (2007), who used gauze soaked in alternatively chlorhexidine and saline to clean implant surfaces. Schou et al. (2003) evaluated different decontamination procedures in an experimental study on treatment of peri-implantitis in monkeys. As no differences were

Table 2. Results from the histometric measurements representing test (chlorhexidine) and control (saline) procedures for implants type A, B, C, D. Mean values and standard deviations (SD) ($n = 6$)

Dimension (mm), area (mm^2)	Implant A		Implant B		Implant C		Implant D	
	Test (Chx)	Control (Saline)	Test (Chx)	Control (Saline)	Test (Chx)	Control (Saline)	Test (Chx)	Control (Saline)
PM-aPE	3.25 (2.15)	2.07 (1.61)	1.92 (0.80)	2.49 (1.58)	2.35 (1.61)	2.24 (1.38)	3.90 (2.08)	4.64 (0.91)
aPE-B	2.23 (0.71)	2.62 (1.21)	2.58 (0.25)	2.74 (1.12)	2.11 (1.10)	2.15 (0.52)	2.37 (2.49)	2.74 (2.12)
Residual Intra-bony Defect Area	4.48 (2.97)	2.61 (4.38)	1.56 (1.09)	3.56 (3.01)	1.47 (1.42)	2.21 (2.62)	5.94 (4.65)	8.89* (2.22)

*$p < 0.05$ between implant D *versus* implant A, B and C of the control group.

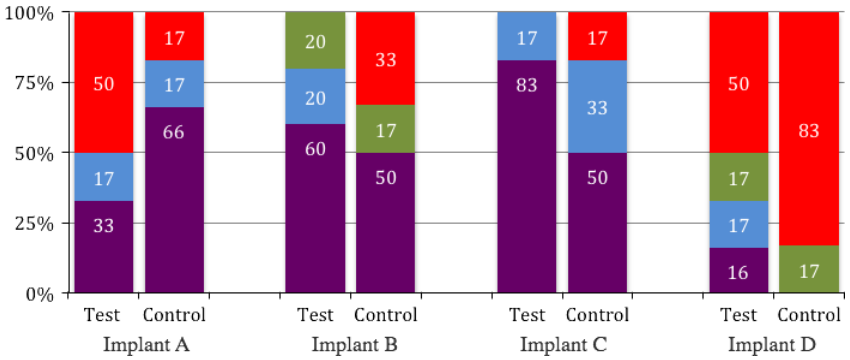

Fig. 4. ICT scores for test (chlorhexidine) and control (saline) sites at implant types A, B, C, D. Score 0 (magenta), 1 (blue), 2 (green), 3 (red).

found between air-powder abrasive procedures, gauze soaked in saline and citric acid or gauze soaked in alternately chlorhexidine and saline in regards to bone fill and re-osseointegration, the authors concluded that the simplest method, that is saline and chlorhexidine soaked gauze, should be used. As the results from this study did not disclose any difference between the use of gauze soaked in saline or chlorhexidine regarding resolution of peri-implantitis lesions, the suggestion by Schou et al. (2003) regarding the simplest method may be restricted to saline. The finding that the use of saline during cleaning of implant surfaces is effective in the resolution of experimental peri-implantitis lesions has been demonstrated previously. Persson et al. (1999) in an experimental study in dogs found no differences between cleaning with saline and the use of abrasive pumice and a rotating brush. While a similar study from the same group (Persson et al. 2001) aimed at evaluating differences in bone fill and re-osseointegration at implants with different surfaces, resolution of peri-implantitis lesion occurred following the local use of pellets soaked in saline at both types of implants. In this context it should be realized that in the studies by Persson et al. (1999, 2001) systemic antibiotics were used as an adjunct to the local treatment procedures. On the other hand, Albouy et al. (2011) in an experimental study in dogs reported on the outcome of treatment of peri-implantitis using gauze soaked in saline and in the absence of systemic antibiotics. Although results varied between different implant types, it was concluded that resolution of peri-implantitis without local and systemic chemical antimicrobial therapy is possible. The finding on the resolution of peri-implantitis lesions reported by Albouy et al. (2011) is supported by observations made in this study.

Although differences between test and control procedures were not statistically significant in the present experiment, implant B, C and D presented with larger mean bone loss for control than for test procedures, whereas a reverse relationship on bone loss was assessed for implant A. The different response to test and control procedures for implant A was not only restricted to bone level changes as matching results were obtained in regards to histological evaluations. The results thus indicated that the use of chlorhexidine on implant A resulted in worse outcomes than the use of saline.

The present experiment also evaluated differences in resolution of peri-implantitis lesions between different types of implants. While the selection of cleaning procedures, that is, saline or chlorhexidine, had minor influence on treatment outcome, the results differed in several aspects between implant types. Thus, results from the longitudinal assessments of bone level changes in radiographs revealed that implants of type C presented with bone gain in both control and test procedures and that bone loss at implant D was significantly larger than at implants A, B and C among control implants. In addition, the microbiological and histological analysis indicated worse results in terms of resolution of peri-implantitis lesions at implants D than implants A, B and C. The findings on different outcomes between implant types following treatment of experimental peri-implantitis are in agreement with data presented in a experimental study in dogs by Albouy et al. (2011). They examined resolution of peri-implantitis following surgical therapy at four different types of implants, out of which two were similar to implants A and D, respectively, of the present experiment. Albouy et al. (2011) reported that implants with a turned surface and those with a TiOblast surface (corresponding to implant A of the present material) presented with bone gain and resolution of peri-implantitis lesions after surgical therapy. In the study by Albouy et al. (2011) it was

Table 3. Changes in total DNA-probe counts ($\times 10^5$) at test (chlorhexidine) and control (saline) groups for each implant type from surgery to 5 months after surgery. Mean values and standard deviation (SD) ($n = 6$)

Total DNA-probe counts changes ($\times 10^5$)	Implant A		Implant B		Implant C		Implant D	
	Test (Chx)	Control (saline)	Test (Chx)	Control (saline)	Test (Chx)	Control (saline)	Test (Chx)	Control (saline)
Day of surgery – 5 months after surgery	−9.97*	−5.8*	−11.69*	−10.83*	−14.9*	−12.6*	−3.47	5.23

*p-value <0.05 between baseline versus 5 months for implant A, B, C.

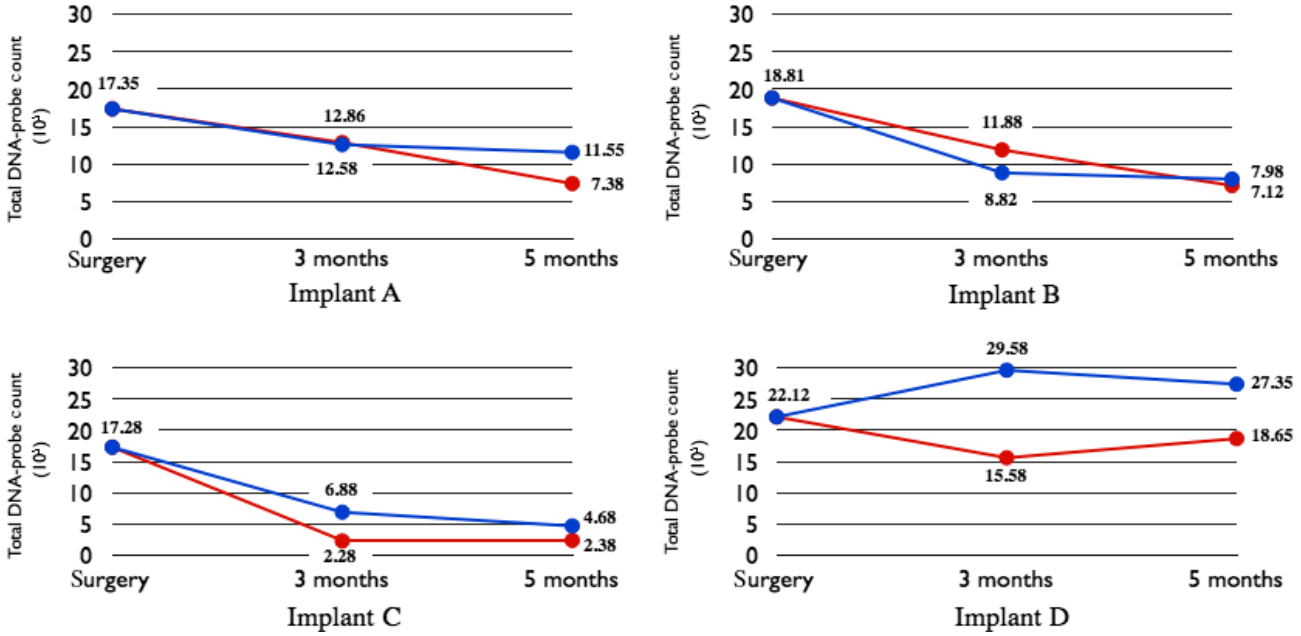

Fig. 5. Total DNA-probe counts changes ($\times 10^5$) after surgical treatment of peri-implantitis for each implant type. Mean values for test (red) and control (blue) sites. ($n = 6$).

also reported that the implants with a TiUnite surface, that is, the category corresponding to implant D of the current experiment, demonstrated additional bone loss and no signs of resolution of peri-implantitis lesions after surgical therapy.

The different results in resolution of peri-implantitis lesions between implant types observed in this study should also be addressed from the perspectives of the retention of the biofilm to the implant surface and obstacles related to the removal of the biofilm. Such problems were addressed in human and in vitro experiments. Henderson et al. (2013) in an in vitro study reported that decontamination of a biofilm that had formed on titanium discs with a smooth surface using solutions of chlorhexidine, saline or EDTA was ineffective, whereas the use of 3% H_2O_2 resulted in reduction in the biofilm. Charalampakis et al. (2014) studied the effect of mechanical and chemical cleansing on an intra-orally formed biofilm on titanium discs with different surface characteristics. Titanium discs representing turned, TiOblast, OsseoSpeed and AT-I surfaces were carried by 20 volunteers for 4 days. The discs were subsequently mechanically cleaned, using cotton pellets soaked in saline, chlorhexidine, delmopinol or essential oils. It was reported that no cleansing method was effective in complete biofilm removal on any of the titanium discs. In addition, the results from the microbiological analysis did not reveal any differences between titanium surface groups or between detergents. The results presented by Charalampakis et al. (2014) in regards to absence of differences between the use of saline or chlorhexidine in the decontamination procedure are in line with data presented in this study, although the cleaning procedure performed by Charalampakis was performed in a more shorter time than in this study. Moreover, three of the surface preparations used in the study by Charalampakis et al. (2014), that is TiOblast, Osseospeed and AT-I, were similar to the implant types A, B and C of the current experiment.

In summary, within the limitations of the present experiment, it is suggested that the local use of chlorhexidine has minor influence on resolution of peri-implantitis following surgical treatment and that the results are influenced by implant surface characteristics.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Bacterial strains used in the study. Abbreviations: CCUG, Culture Collection University of Gothenburg. ATCC, American Culture Collection, OMGS, Oral Microbiology, Gothenburg, Sweden.

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Clinical Relevance

Scientific rationale for the study: Models of experimental peri-implantitis are fundamental for the research on treatment of the disease. While implant surface decontamination procedures in previous experiments on treatment of peri-implantitis often included the use of gauze soaked in chlorhexidine or saline, the effect on resolution of peri-implantitis lesions was rarely addressed. In addition, few studies evaluated the influence of implant surface characteristics on treatment outcomes.

Principle findings: It was demonstrated that (i) the local use of chlorhexidine has minor influence on treatment outcome, (ii) resolution of peri-implantitis following surgical treatment without the adjunctive use of local and systemic antimicrobial agents is possible and (iii) the results are influenced by implant surface characteristics.

Practical implications: The results of the present experiment indicate that peri-implantitis treatment outcome may be different for various types of implants.

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Adjunctive Systemic and Local Antimicrobial Therapy in the Surgical Treatment of Peri-implantitis: A Randomized Controlled Clinical Trial

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Abstract

The aim of the present randomized controlled clinical trial was to investigate the adjunctive effect of systemic antibiotics and the local use of chlorhexidine for implant surface decontamination in the surgical treatment of peri-implantitis. One hundred patients with severe peri-implantitis were recruited. Surgical therapy was performed with or without adjunctive systemic antibiotics or the local use of chlorhexidine for implant surface decontamination. Treatment outcomes were evaluated at 1 y. A binary logistic regression analysis was used to identify factors influencing the probability of treatment success, that is, probing pocket depth ≤5 mm, absence of bleeding/suppuration on probing, and no additional bone loss. Treatment success was obtained in 45% of all implants but was higher in implants with a nonmodified surface (79%) than those with a modified surface (34%). The local use of chlorhexidine had no overall effect on treatment outcomes. While adjunctive systemic antibiotics had no impact on treatment success at implants with a nonmodified surface, a positive effect on treatment success was observed at implants with a modified surface. The likelihood for treatment success using adjunctive systemic antibiotics in patients with implants with a modified surface, however, was low. As the effect of adjunctive systemic antibiotics depended on implant surface characteristics, recommendations for their use in the surgical treatment of peri-implantitis should be based on careful assessments of the targeted implant (ClinicalTrials.gov NCT01857804).

Keywords: dental implant, amoxicillin, implant surface decontamination, radiographs, logistic regression, treatment success

Introduction

Peri-implantitis is a pathological condition occurring in patients with dental implants and is characterized by inflammation in peri-implant tissues and loss of supporting bone. As peri-implantitis is caused by bacteria, the treatment of the disease should include anti-infective measures, and the goals of therapy should include disease resolution and preservation of supporting bone (Lindhe and Meyle 2008).

Surgical therapy is required in the treatment of peri-implantitis to promote access for debridement of contaminated implant surfaces. The use of different decontamination procedures has included mechanical and chemical techniques, but no single method or combination of methods has been shown to be superior (Lindhe and Meyle 2008; Renvert et al. 2012). Adjunctive systemic antibiotic regimens were frequently applied in case series on the surgical treatment of peri-implantitis without evaluating their potential benefit (Graziani et al. 2012; Renvert et al. 2012). Results from preclinical in vivo studies on the surgical treatment of experimental peri-implantitis, however, demonstrated that resolution of the disease is possible in the absence of adjunctive systemic and local antimicrobial therapy (Albouy et al. 2011; Carcuac et al. 2015).

Results from retrospective studies on the surgical therapy of peri-implantitis indicated varying degrees of successful outcomes (Charalampakis et al. 2011; Lagervall and Jansson 2013). The discrepancy in the onset and progression of the disease among patients and the large variation in treatment methods of surgical therapy of peri-implantitis, however, hampered analyses and conclusions.

The quality of reporting in clinical studies on the treatment of peri-implantitis was assessed in a systematic review by Graziani et al. (2012). It was reported that the literature is based on studies using small sample sizes with short-term follow-up and a diversity of interventions tested. A consensus report from the 8th European Workshop on Periodontology emphasized the need for identifying a standard mode of therapy for the treatment of peri-implantitis (Sanz and Chapple 2012). It was concluded that randomized controlled clinical trials are

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A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

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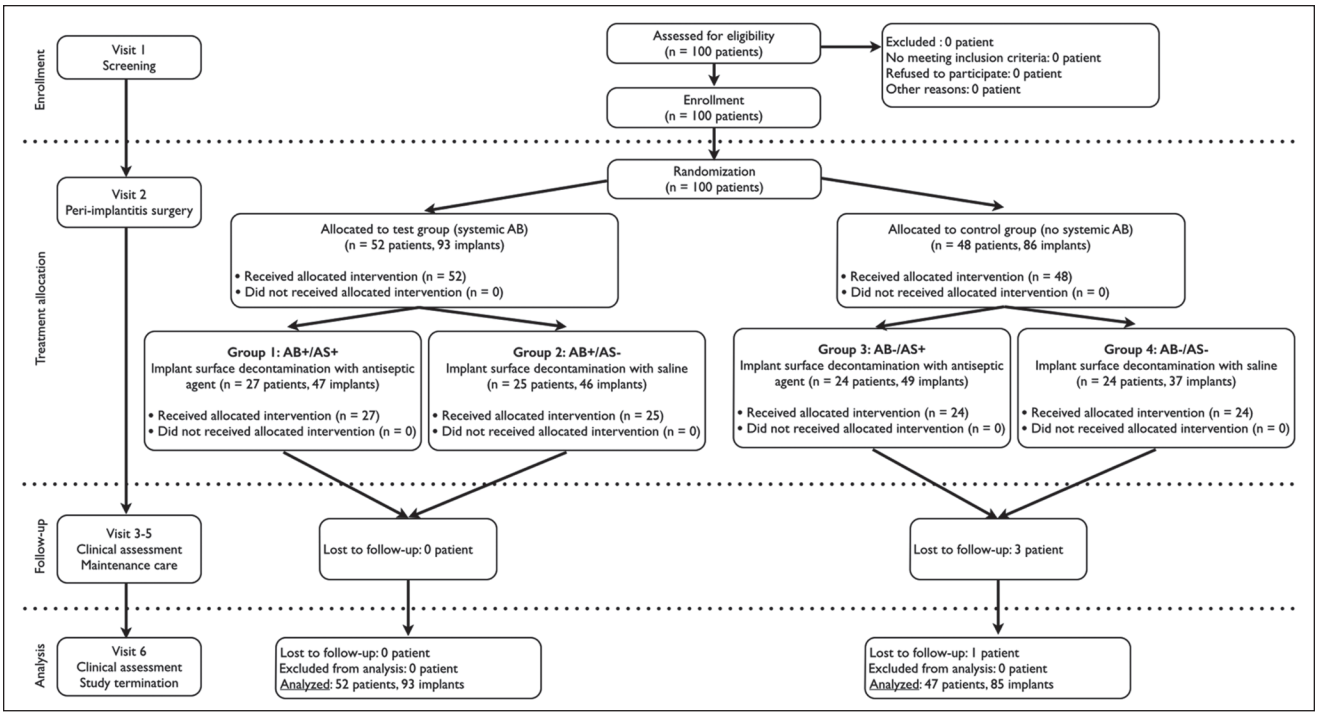

Figure 1. CONSORT flowchart of the study.

needed to test the hypothesis that adjunctive systemic antimicrobial therapy enhances treatment outcomes of the surgical therapy of peri-implantitis and that such parallel-arm, randomized controlled clinical trials should include an end-point assessment of at least 6 and 12 mo. The consensus report also recommended that a composite outcome of disease resolution should be used. This composite outcome should include an absence of deep probing pockets with bleeding or suppuration and no further bone loss (Sanz and Chapple 2012).

This study reports on a 1-y follow-up of patients enrolled in a prospective randomized controlled clinical trial aimed at investigating the adjunctive effect of systemic antibiotics and the local use of chlorhexidine for implant surface decontamination in the surgical treatment of peri-implantitis.

Materials and Methods

Patient Selection

The study was registered at ClinicalTrials.gov (NCT01857804) and approved by the Regional Ethical Committee, Gothenburg, Sweden (Dnr. 654-10). All subjects were informed about the study, given a detailed description of the procedure, and signed a written consent form. CONSORT (Consolidated Standards of Reporting Trials) guidelines for clinical trials were followed, and the study flowchart is presented in Figure 1.

The study population consisted of 100 patients (35 males and 65 females; mean age, 66.3 ± 13.6 y) presenting with severe peri-implantitis in ≥1 implants (i.e., peri-implant probing pocket depth [PPD] ≥6 mm in at least 1 aspect of the implant, together with bleeding and/or suppuration on probing [BoP and/or SoP, respectively] and radiographically documented marginal bone loss >3 mm). The patients were referred to 2 specialist clinics in periodontics (Mölndal and Gothenburg, Public Dental Health Services, Region Västra Götaland, Sweden) and were enrolled between October 2010 and December 2013. Exclusion criteria were compromised general health, systemic antibiotic therapy during the past 6 mo, and allergy to penicillin.

Baseline Examination and Randomization Procedure

In the baseline examination, the following variables were recorded at the mesial, distal, buccal, and lingual aspects of each implant: PPD measured with a manual periodontal probe (Hu-Friedy, Chicago, IL, USA) and bleeding/suppuration within 15 s following pocket probing.

Patients were randomly allocated to 4 treatment groups using computer-generated lists: group 1: systemic antibiotics/implant surface decontamination with an antiseptic agent (n = 27); group 2: systemic antibiotics/implant surface decontamination with saline (n = 25); group 3: no systemic antibiotics/implant surface decontamination with an antiseptic agent (n = 24); and group 4: no systemic antibiotics/implant surface decontamination with saline (n = 24).

The allocation procedure was stratified for smokers/non-smokers. Patient and implant data are presented in Table 1. The 100 patients presented with 179 affected implants, of which

Table 1. Demographic Data on Patients and Characteristics of Affected Implants.

	All Groups	Group 1 (AB+/AS+)	Group 2 (AB+/AS–)	Group 3 (AB–/AS+)	Group 4 (AB–/AS–)
Number of patients	100	27	25	24	24
Age, mean (range), y	66.3 (21–90)	65.7 (23–90)	67.9 (21–88)	64.6 (27–81)	66.9 (30–88)
Gender, *n* (%)					
Male	35	7 (25.9)	8 (32)	10 (41.7)	10 (41.7)
Female	65	20 (74.1)	17 (68)	14 (58.3)	14 (58.3)
Smoking habit,[a] *n* (%)					
Smoker	33	9 (33.3)	9 (36)	8 (33.3)	7 (29.2)
Nonsmoker	67	18 (66.7)	16 (64)	16 (66.7)	17 (70.8)
History of periodontitis,[b] *n* (%)	84	21 (77.8)	21 (84)	21 (87.5)	21 (87.5)
Diabetes,[a] *n* (%)	5	2 (7.4)	0	1 (4.2)	2 (8.3)
CVD-related drug therapy,[a] *n* (%)	31	9 (33.3)	8 (32)	6 (25)	8 (33.3)
Number of implants presenting with peri-implantitis (range)	179 (1–7)	47 (1–5)	46 (1–6)	49 (1–7)	37 (1–6)
Jaw, *n* (%)					
Maxilla	116 (64.8)	35 (74.5)	28 (60.9)	32 (65.3)	21 (56.8)
Mandible	63 (35.2)	12 (25.5)	18 (39.1)	17 (34.7)	16 (43.2)
Location, *n* (%)					
Anterior (incisor-canine)	91 (50.8)	25 (53.2)	23 (50)	26 (53.1)	17 (45.9)
Posterior (premolar-molar)	88 (49.2)	22 (46.8)	23 (50)	23 (46.9)	20 (54.1)
Implant surface category,[c] *n* (%)					
Nonmodified					
A	43 (24)	3 (6.4)	12 (26.1)	15 (30.6)	13 (35.1)
Modified					
All modified	136 (76)	44 (93.6)	34 (73.9)	34 (69.4)	24 (64.9)
B	87	30	21	26	10
C	9	2	2	1	4
D	24	7	6	4	7
E	13	5	5	1	2
F	3	0	0	2	1

AB, antibiotic; AS, antiseptic; CVD, cardiovascular disease.
[a]Self-reported information was used for the assessment of smoking habit, presence of diabetes, and CVD-related drug therapy.
[b]The presence of approximal attachment loss exceeding 2 mm in ≥2 teeth, as assessed by radiographs and by clinical examination, was scored as a history of periodontitis.
[c]A: turned surface (Nobel Biocare AB, Göteborg, Sweden); B: TiUnite surface (Nobel Biocare AB); C: TiOblast surface (Astra Tech Implant System; Dentsply Implant IH AB, Mölndal, Sweden); D: OsseoSpeed surface (Astra Tech Implant System); E: SLA surface (Straumann; Institute Straumann, Basel, Switzerland); F: Neoss ProActive surface (Neoss Ltd., Harrogate, UK).

51% were placed in an anterior position and 65% were located in the maxilla. Twenty-four percent of all implants had a nonmodified surface (category A). In patient groups 1 and 2, the 10-d systemic antibiotic regimen (amoxicillin 2 × 750 mg daily) commenced 3 d prior to surgery. In patient groups 1 and 3, an antiseptic agent (0.2% solution of chlorhexidine digluconate [CHX]) was applied for implant surface decontamination during surgery.

Sample size calculation was based on a difference of PPD reduction between groups of 0.5 mm with a standard deviation (SD) of 0.5 mm, a significance level of 5%, and 80% power. The required sample size was 20 subjects for each treatment group.

Microbiological Sampling and Analysis

Samples from subgingival microbiota were obtained from implant sites targeted for surgical therapy. The sampling area was isolated with cotton rolls and dried, and supragingival plaque on the implants was removed with sterile cotton pellets. Six sterile paper points (size 35; Dentsply Maillefer, Ballaigues, Switzerland) were inserted into the most apical part of the peri-implant pocket, kept in place for 10 s, and then placed in 2 different tubes for culture and checkerboard DNA-DNA hybridization analyses, respectively. For details regarding checkerboard DNA-DNA hybridization and culture techniques, see the Appendix and Charalampakis et al. (2011).

Surgical Procedure

Prior to surgery, patients were enrolled in a hygiene program including professional supragingival implants/teeth cleaning using rubber cups, polishing paste, and oral hygiene instructions. The surgical procedure was aimed at pocket elimination using resective techniques. Surgeries were performed by 5 experienced periodontists (O.C., J.D., I.A., J.W., and T.B.). Screw-retained supraconstructions were removed. Following local anesthesia, intrasulcular incisions were performed, and full-thickness flaps were elevated on the buccal and lingual aspects of affected implants. Inflamed tissue was removed, and

titanium-coated curettes (Hu-Friedy) were used to remove hard deposits on implants. Implant surfaces were decontaminated with 10 × 10–mm gauze soaked in either 0.2% CHX (groups 1 and 3) or saline (groups 2 and 4) for 2 min. Osseous recontouring was performed when indicated, and flaps were adjusted and closed with single interrupted sutures. Supraconstructions were reconnected. Patients rinsed for 1 min with 0.2% CHX twice daily for 14 d following surgery.

Sutures were removed 2 wk after surgical therapy, and self-performed mechanical infection control procedures were initiated. Intraoral radiographs were obtained using a long-cone paralleling technique and a digital radiography sensor (74321; Dürr Dental AG, Bietigheim-Bissingen, Germany) with a sensor holder (Eggen-holder/Super-Bite blocks; Kerr Dental, Orange, CA, USA). The radiographs were analyzed with image software (ImageJ64; National Institutes of Health, Bethesda, MD, USA). The known interthread pitch distance of the implant was used in each radiograph for calibration of the coronal-apical measurements. The marginal bone level was assessed at the mesial and distal aspects of each implant at ×10 magnification on a high-definition monitor. All radiological assessments were performed by 1 investigator (O.C.).

Evaluation at 6 and 12 mo following Treatment

During the 12-mo follow-up period, supragingival polishing was performed and oral hygiene reinforced in 3-mo intervals. Microbiological samples were taken at 3, 6, and 12 mo after surgery. At 6 and 12 mo, clinical assessments of PPD, BoP, and SoP were performed. In addition, new intraoral radiographs were obtained at the 12-mo examination. Adverse events throughout the study period were also recorded.

Bone level changes between 2 wk and 12 mo after surgery were assessed. For validation of bone level measurements, the radiographs of 30 patients were randomly selected and remeasured by 2 investigators (O.C. and J.D.). Double measurements revealed an interexaminer agreement (interclass correlation) of 0.97, with a mean (±SD) difference between the 2 observers of 0.37 ± 0.49 mm. For the intraexaminer agreement, the corresponding values were 0.98, with a mean of 0.35 ± 0.22 mm.

Data Analysis

Clinical variables at baseline and 6 and 12 mo were expressed in mean values and frequency distributions (SPSS 21.0 software package; SPSS Inc., Chicago, IL, USA). Differences were analyzed using analysis of variance, the χ^2 test (between groups), and the McNemar test (within groups). Adjustment for multiple comparisons (pairwise tests) was performed using the Bonferroni correction method. A P value <0.05 was considered as significant.

Implant sites presenting with a PPD ≤5 mm, absence of BoP/SoP at the 12-mo examination, and bone loss ≤0.5 mm between 2 wk and 12 mo after surgical therapy were considered as a treatment success and the primary outcome variable. To identify factors affecting the probability of treatment success, a multiple logistic multilevel model (*xtlogit* in Stata Statistical Software Release 13; StataCorp LP, College Station, TX, USA) was used. The hierarchical analysis included the patient at the higher level and the implant at the lower level. The logit function was applied to link the linear model with the probability of the binary event. The independent factors examined included treatment factors, patient-related data (age, gender, smoking habit, history of periodontitis, and systemic disorder), and implant-related data (number of affected implants, jaw, and location). Implants were further categorized according to surface characteristics (nonmodified and modified). The model was built with the intercept as a random term. All variables were assessed by the Wald test in a bivariate analysis, and only statistically significant variables ($P < 0.05$) were retained in the multiple model. The 2 treatment factors were forced into the final model, and a possible interaction between factors was explored. Results were expressed as odds ratios (ORs) including 95% confidence intervals (CIs).

Results

Three patients (2 patients in group 3 and 1 patient in group 4) did not undergo the examination at 6 mo after surgery but attended the final examination (12 mo). One patient with 1 affected implant in group 3 did not undergo the examination at 6 and 12 mo. All patients in groups 1 and 2 reported complete adhesion to the systemic antibiotic regimen. Five of these patients reported mild gastrointestinal problems. During the 1-y follow-up period, 6 implants in 6 patients were disintegrated and hence removed (group 1: 1 implant/1 patient; group 3: 3 implants/3 patients; and group 4: 2 implants/2 patients). All lost implants had a modified surface.

Reduction in PPD occurred in all treatment groups but was significantly larger in group 2 than in groups 3 and 4 at the 1-y examination. At 6 mo following the surgical treatment of peri-implantitis, BoP remained at 53% in affected implants. Further improvement (42%) was observed at 12 mo, with no significant differences between treatment groups. At 12 mo, SoP was observed in 17% of all sites. Bone gain was observed in implants in patients of groups 1 and 2, while additional bone loss occurred in the other 2 groups (Table 2).

The overall profile of changes in total DNA probe counts was similar for the 4 treatment protocols and exhibited a significant decline during the 12-mo period after surgical therapy (Appendix Fig.). The total viable count (TVC) also decreased after surgery in all treatment groups. Checkerboard and culture analyses showed that *Fusobacterium nucleatum* and *Prevotella intermedia/Prevotella nigrescens* were the most common types of bacteria presenting moderately heavy/heavy growth at baseline (71% and 46% of the patients, respectively) and 1 y after surgical treatment (54% and 43% of the patients, respectively). Moderately heavy/heavy growth of *Staphylococcus aureus* was detected in 1 patient before surgery but not at the 1-y examination. No patient presented with moderately heavy/heavy growth of *Aggregatibacter actinomycetemcomitans*.

Table 2. Results from Clinical and Radiological Examinations.

	All Groups	Group 1 (AB+/AS+)	Group 2 (AB+/AS–)	Group 3 (AB–/AS+)	Group 4 (AB–/AS–)
Probing pocket depth at deepest site at baseline, mm	7.82 ± 1.52	7.85 ± 1.57	7.93 ± 1.50	7.79 ± 1.69	7.78 ± 1.25
Probing depth changes, mm					
Baseline to 6 mo	–2.71 ± 1.71	–3.03 ± 1.58[a]	–3.49 ± 1.54[b]	–2.18 ± 1.54[b]	–1.95 ± 1.81[a,b]
Baseline to 1 y	–2.58 ± 1.97	–2.80 ± 1.87	–3.44 ± 1.66[b]	–2.16 ± 1.79[b]	–1.69 ± 2.22[b]
Bleeding on probing, *n* (%)					
6 mo	92 (52.9)	16 (34)[a]	24 (52.2)	26 (56.5)	26 (74.3)[a]
1 y	72 (41.9)	18 (39.1)	16 (34.8)	20 (44.4)	18 (51.4)
Suppuration on probing, *n* (%)					
Baseline	123 (68.7)	34 (72.3)	30 (65.2)	33 (67.3)	26 (70.3)
6 mo	25 (14.4)	5 (10.6)	2 (4.3)[c]	9 (19.6)	9 (25.7)[c]
1 y	30 (17.4)	6 (13)	3 (6.5)[c]	10 (22.2)	11 (31.4)[c]
Bone level changes between 2 wk and 12 mo after surgery, mm	–0.21 ± 1.32	0.18 ± 1.15[d]	0.51 ± 0.84[d]	–0.69 ± 1.32[d]	–0.96 ± 1.42[d]

At baseline (n = 179) and 6 (n = 174) and 12 mo (n = 172) after surgical treatment. Values are shown as mean ± standard deviation unless otherwise indicated. AB, antibiotic; AS, antiseptic.
[a]$P < 0.05$ (group 1 v. group 4).
[b]$P < 0.05$ (group 2 v. groups 3 and 4).
[c]$P < 0.05$ (group 2 v. group 4).
[d]$P < 0.05$ (groups 1 and 2 v. groups 3 and 4).

Details from checkerboard and culture analyses are presented in the Appendix Table.

Treatment success was achieved in 45% of all implants at 12 mo after surgical therapy. The corresponding value assessed at the patient level was 38% (Table 3). Treatment success was obtained overall in 79.1% of the implants and in 66.7% of the patients representing implant surface category A (nonmodified surface). The corresponding data for implants with modified surfaces (categories B, C, D, E, and F) were 34.1% and 32.5%, respectively. In addition, the absence of the adjunctive use of systemic antibiotics or local antiseptics had a minor effect on treatment success for implant category A. In implant category B, however, no cases exhibited treatment success in the absence of systemic antibiotics (treatment groups 3 and 4) (Table 3). Clinical and radiological results of 2 patients are presented in Figure 2.

The local use of antiseptics had no overall effect on treatment success (OR, 0.31; $P = 0.209$), while cardiovascular disease (CVD)–related drug therapy negatively affected outcomes (OR, 0.11; $P = 0.039$) (Table 4). The analysis demonstrated an interaction between the effects of adjunctive antibiotics and surface characteristics. Thus, the use of systemic antibiotics had no impact on treatment success at implants with a nonmodified surface (OR, 0.27; $P = 0.506$), whereas at implants with a modified surface, a positive effect on treatment success was observed (OR, 38.69; $P = 0.005$). Consequently, in the absence of systemic antibiotics, implants with a modified surface showed significantly lower odds (OR, 0.002; $P = 0.002$) for treatment success compared to implants with a nonmodified surface.

Based on data presented in Table 3, the number of patients needed to be treated with adjunctive systemic antibiotics to obtain treatment success at implants with a modified surface was 5 (95% CI, 2.3–23.8; absolute risk reduction = 23.74%).

Discussion

The present randomized controlled clinical trial evaluated the adjunctive effect of systemic antibiotics and the local use of chlorhexidine for implant surface decontamination in the surgical treatment of peri-implantitis. It was demonstrated that treatment success was obtained in 45% of all implants but was higher in implants with a nonmodified surface (79%) than those with a modified surface (34%). The local use of chlorhexidine had no overall effect on treatment outcomes. While adjunctive systemic antibiotics had no impact on treatment success in implants with a nonmodified surface, a positive effect on treatment success was observed at implants with a modified surface. The likelihood for treatment success using adjunctive systemic antibiotics in patients with implants with modified surfaces, however, was low.

The evaluation of outcomes in the present study was confined to treatment success criteria that included the combination of findings from clinical and radiological assessments. A similar composite outcome was not applied in previous prospective studies on ≥1-y follow-up after the surgical treatment of peri-implantitis using pocket elimination procedures. Serino and Turri (2011) evaluated results at 2 y after the surgical therapy of peri-implantitis in 31 patients. It was reported that surgical treatment together with the adjunctive use of systemic antibiotics was an effective therapy for the majority of cases but that results depended on the initial severity of the disease. While no data on bone level changes after surgery were presented by Serino and Turri (2011), data from the 2-y examination disclosed that 48% of the patients had no implant sites with BoP or SoP. In a study on 24 patients, Heitz-Mayfield et al. (2012) reported on results from a 1-y follow-up after the surgical therapy of peri-implantitis with the adjunctive use of systemic antibiotics. It was reported that the mean PPD was <5 mm with an absence

Table 3. Treatment Success.

	Implant Level (n = 178)								Patient Level (n = 99)							
		Implant Surface Category[a]								Implant Surface Category[a]						
		Nonmodified	Modified							Nonmodified	Modified					
	All Implants	A	All Modified	B	C	D	E	F	All Patients	A	All Modified	B	C	D	E	F
All groups	80/178 (44.9)	34/43 (79.1)	46/135 (34.1)	14/86	4/9	16/24	11/13	0/3	38/99 (38.4)	12/19 (66.7)	26/80 (32.5)	6/47	4/7	11/17	5/7	0/2
Group 1 (AB+/AS+)	19/47 (40.4)	1/3 (33.3)	18/44 (40.9)	6/30	1/2	6/7	5/5	—	10/27 (37)	1/3 (33.3)	9/24 (37.5)	4/17	0/1	4/5	1/1	—
Group 2 (AB+/AS–)	30/46 (65.2)	10/12 (83.3)	20/34 (58.8)	8/21	2/2	6/6	4/5	—	14/25 (56)	4/5 (80)	10/20 (50)	2/11	2/2	4/4	2/3	—
Group 3 (AB–/AS+)	18/48 (37.5)	14/15 (93.3)	4/33 (12.1)	0/26	1/1	3/4	0/1	0/2	7/23 (30.4)	4/5 (80)	3/18 (16.7)	0/12	1/1	2/3	0/1	0/1
Group 4 (AB–/AS–)	13/37 (35.1)	9/13 (69.2)	4/24 (16.7)	0/10	1/4	1/7	2/2	0/1	7/24 (29.2)	3/6 (50)	4/18 (22.2)	0/7	1/3	1/5	2/2	0/1

Success is defined as implants presenting with a probing pocket depth ≤5 mm, absence of bleeding/suppuration on probing, and bone loss ≤0.5 mm. Values are shown as number of implants/patients (%). AB, antibiotic; AS, antiseptic.
[a]A: turned surface (Nobel Biocare AB, Göteborg, Sweden); B: TiUnite surface (Nobel Biocare AB); C: TiOblast surface (Astra Tech Implant System; Dentsply Implant IH AB, Mölndal, Sweden); D: OsseoSpeed surface (Astra Tech Implant System); E: SLA surface (Straumann; Institute Straumann, Basel, Switzerland); F: Neoss ProActive surface (Neoss Ltd., Harrogate, UK).

Table 4. Multiple Multilevel Analysis of Factors Associated with Treatment Success.

	OR	95% CI	*P* Value
Antibiotics			
No	1	—	—
Yes	0.27	0.005–12.99	0.506
Antiseptics			
No	1	—	—
Yes	0.31	0.05–1.93	0.209
CVD-related drug therapy			
No	1	—	—
Yes	0.11	0.15–0.90	0.039
Implant surface modification			
Nonmodified	1	—	—
Modified	0.002	0.00005–0.11	0.002
Interaction of antibiotics (yes) × implant surface modification (modified)	144.37	1.12–18,510.09	0.045

CI, confidence interval; CVD, cardiovascular disease; OR, odds ratio.

of BoP in 47% of the implants and that bone levels were unchanged or exhibited bone gain in 92% of implants after treatment. The reduction of PPD and percentage of BoP after the treatment of peri-implantitis reported in the studies by Serino and Turri (2011) and Heitz-Mayfield et al. (2012) are in agreement with data obtained in the present study.

Assessments of bone loss in radiographs require threshold values that consider the measurement error. Thus, the threshold of 0.5 mm used as 1 of the 3 treatment success criteria in the present study was justified as the measurement error was small and in line with results presented in observational studies in the field (Pikner et al. 2009; Koldsland et al. 2010).

The results from the multiple multilevel analysis of factors influencing the probability for treatment success indicated that the local use of chlorhexidine during surgery did not influence the overall probability for treatment success. On the other hand, patients on CVD-related drug therapy presented with significantly lower odds for treatment success. While the relevance in regards to patients with a history of CVD and treatment of peri-implantitis is unclear, findings from risk assessments for peri-implantitis in a case-control study by Renvert et al. (2014) indicated that a history of CVD confers a larger risk for peri-implantitis than a history of periodontitis.

The analysis in the present study also revealed that the odds for treatment success for implants with modified surfaces were significantly lower than for those with a nonmodified surface. Roccuzzo et al. (2011) evaluated the treatment of peri-implantitis using reconstructive procedures in implants with either a rough (TPS) or a moderately rough (SLA) surface in 26 patients and reported that the reduction of PPD and percentage of BoP were more pronounced at implants with a moderately rough surface. Similar observations were made in preclinical in vivo studies. Albouy et al. (2011), in an experimental study in dogs, examined outcomes following the surgical treatment of peri-implantitis at 4 different types of implants (nonmodified surface, SLA, TiOblast, and TiUnite). Surgical therapy was performed,

and after a 6-mo healing period, bone gain and a resolution of inflammation occurred in implants with a nonmodified surface and in TiOblast and SLA surfaces, whereas bone loss occurred in implants with the TiUnite surface. In a similar study in dogs, Carcuac et al. (2015) reported that results were influenced by implant surface characteristics. The findings in the studies by Albouy et al. (2011) and Carcuac et al. (2015) regarding implants with a TiUnite surface may be addressed with respect to implants of surface category B in the present study. This group of implants represented 49% of the entire sample. Furthermore, they exhibited the lowest overall frequency of implants/patients with treatment success (16% and 13%, respectively) and had no cases with treatment success when treatment protocols without adjunctive systemic antibiotics (groups 3 and 4) were used. The observed lack of effect of the local use of chlorhexidine on treatment outcomes reported in the study by Carcuac et al. (2015) is also consistent with findings in the present investigation.

The evaluation protocol of the present study also included microbiological assessments following surgical therapy. Although changes in the TVC between treatment groups were less consistent with changes in total DNA probe counts, overall microbiological outcomes appeared to be independent of the use of adjunctive systemic antibiotics and/or local antiseptics. In this context, it should also be noted that the occurrence of *S. aureus* was limited to 1 patient at baseline and that moderately heavy/heavy growth of *A. actinomycetemcomitans* was never detected. de Waal et al. (2013) reported on changes in clinical and microbiological outcomes over 12 mo following the surgical therapy of peri-implantitis in 30 patients. Although the use of a combination of detergents resulted in a greater immediate suppression of anaerobic bacteria than a placebo procedure, no differences were detected in clinical outcomes. The findings reported in the study by de Waal et al. (2013) are partly in agreement with data presented in the current investigation, as the local use of chlorhexidine influenced neither clinical nor microbiological outcomes.

In summary, the present randomized controlled clinical trial demonstrated that the local use of chlorhexidine had no overall effect on treatment outcomes and that implants with a modified surface showed significantly lower odds for treatment success. As the effect of adjunctive systemic antibiotics depended on implant surface characteristics, recommendations for their use in the surgical treatment of peri-implantitis should be based on careful assessments of the targeted implant.

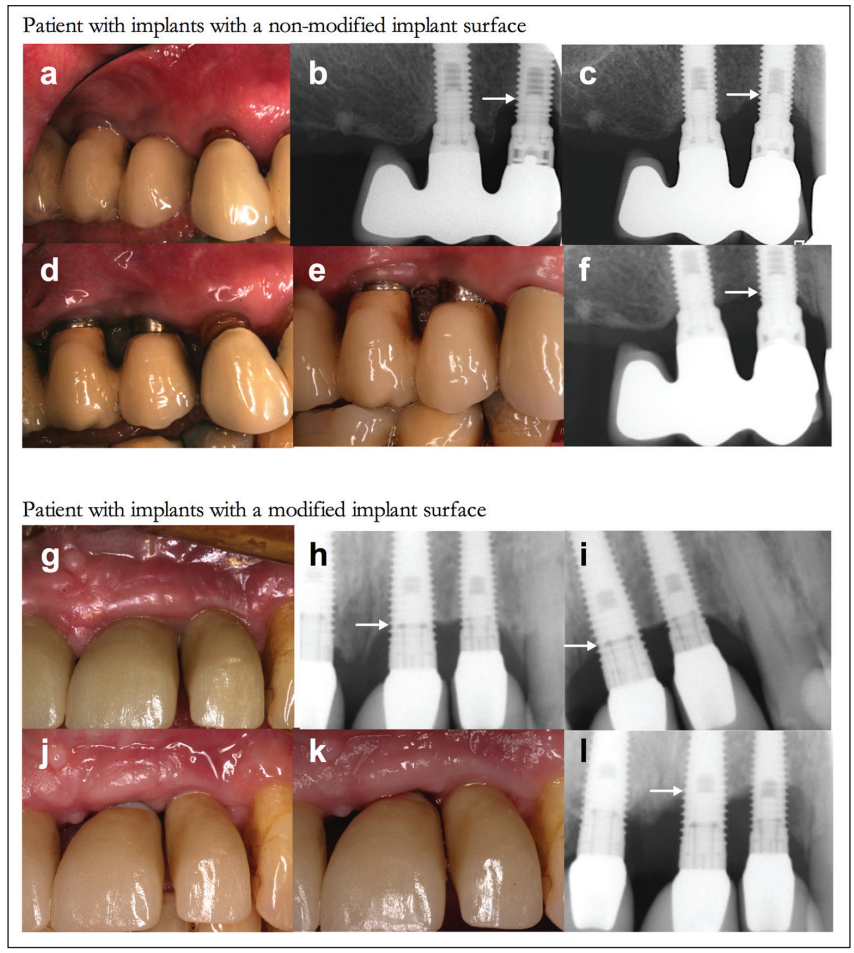

Figure 2. Patient with implants with a nonmodified implant surface (**A–F**). Clinical and radiological documentation at baseline examination (A, B). Radiographs at 2 wk after surgery (C). Clinical image at 6 mo (D) and 1 y after surgical therapy (E). Radiograph at 1 y after surgical therapy (F). Patient with implants with a modified implant surface (**G–L**). Clinical and radiological documentation at baseline examination (G, H). Radiographs at 2 wk after surgery (I). Clinical image at 6 mo (J) and 1 y after surgical therapy (K). Radiograph at 1 y after surgical therapy (L). Arrows indicate marginal bone levels.

Author Contributions

O. Carcuac, T. Berglundh, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; J. Derks, contributed to data acquisition, analysis, and interpretation, critically revised the manuscript; G. Charalampakis, contributed to data analysis and interpretation, critically revised the manuscript; I. Abrahamsson, J. Wennström, contributed to data acquisition, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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