Peri-implantitis and periodontitis
Experimental and clinical studies

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Cover illustration: radiographs, clinical images and histological sections (immunohisto-chemical 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 photosensitization.	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 systemic 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**)

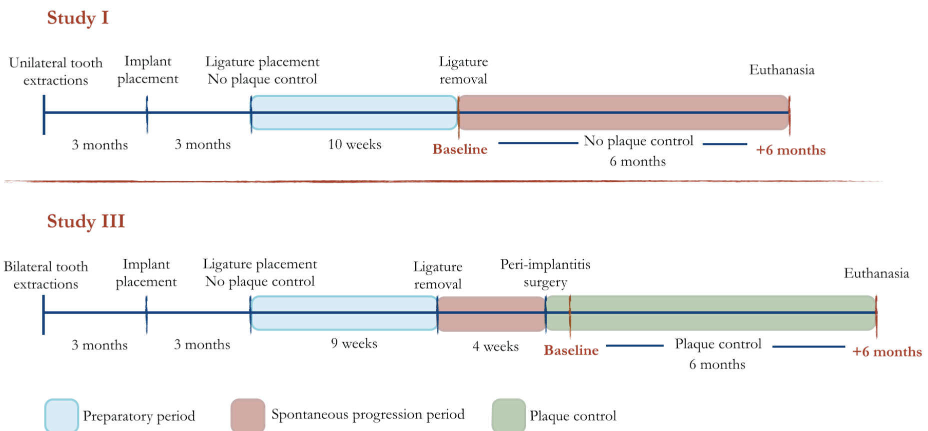
Material & methods

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.



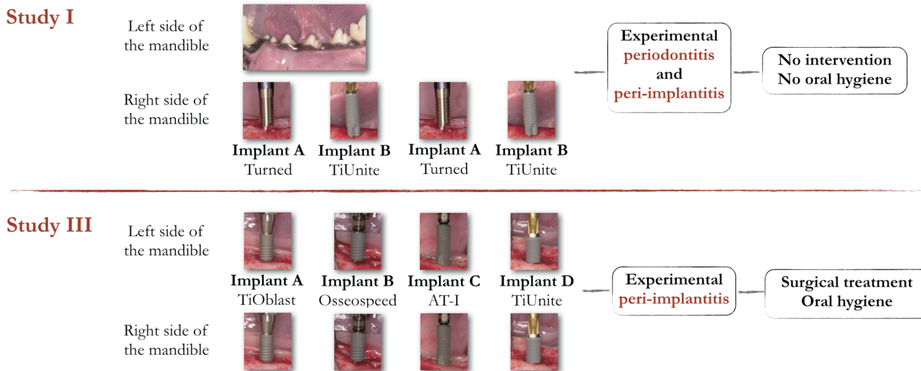
General anesthesia

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^{TM} 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^{TM} surface (implant A), an Osseospeed^{TM} 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^{TM} 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.

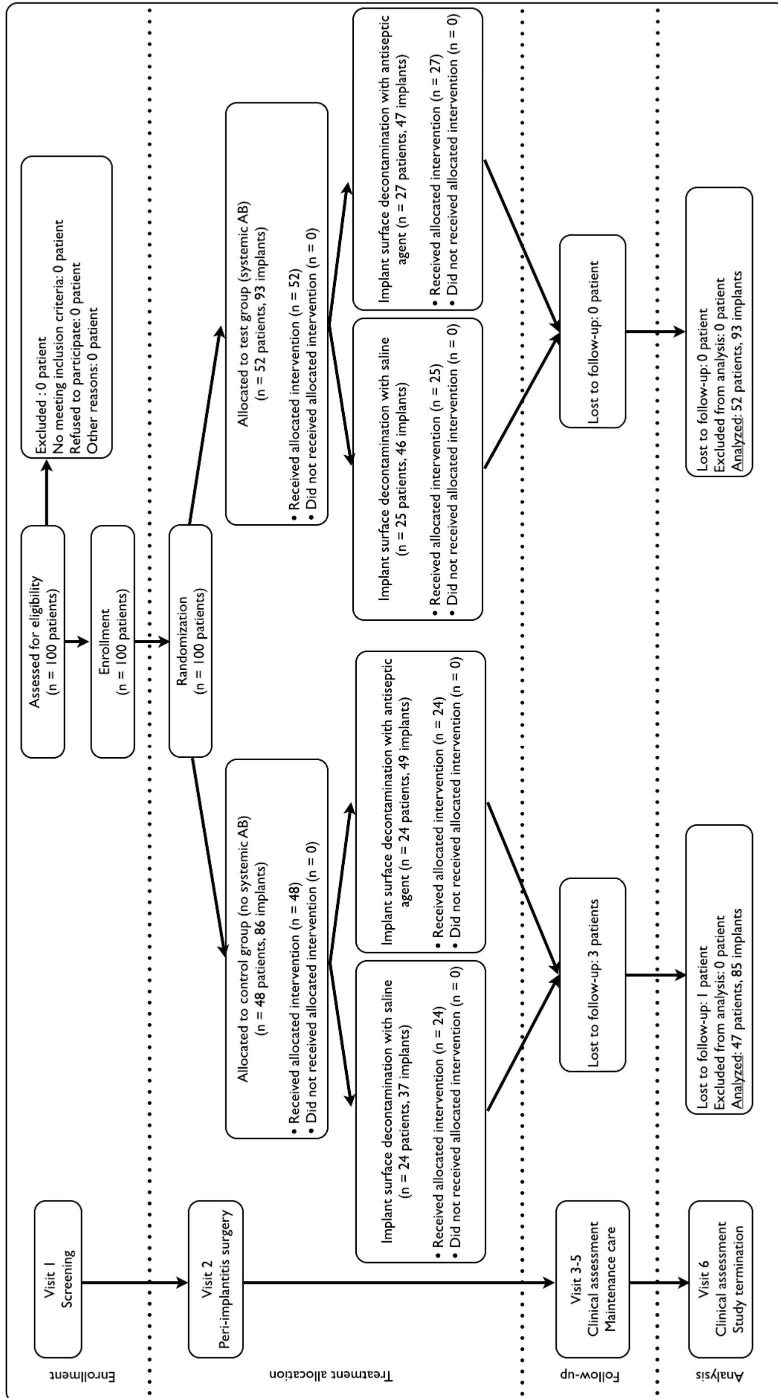


Table 5. Demographic data on patients.

		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

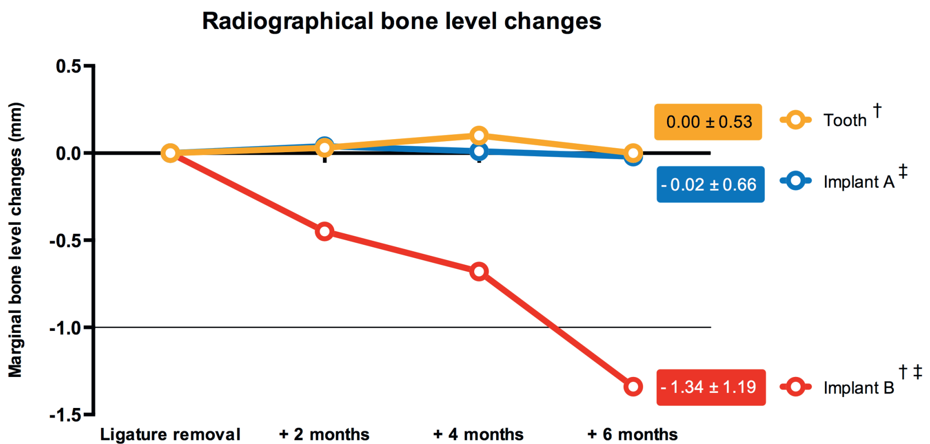
Comparison peri-implantitis/periodontitis (Study I and II)

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

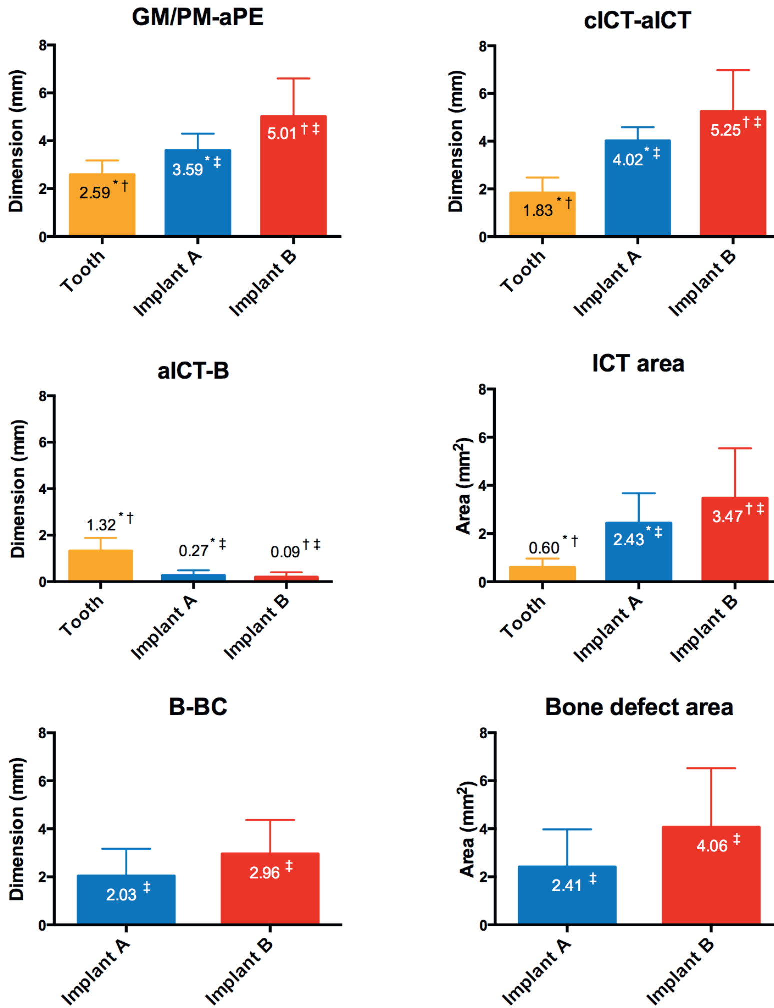
The results from the immunohistochemical analysis are illustrated in Table 9.

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.

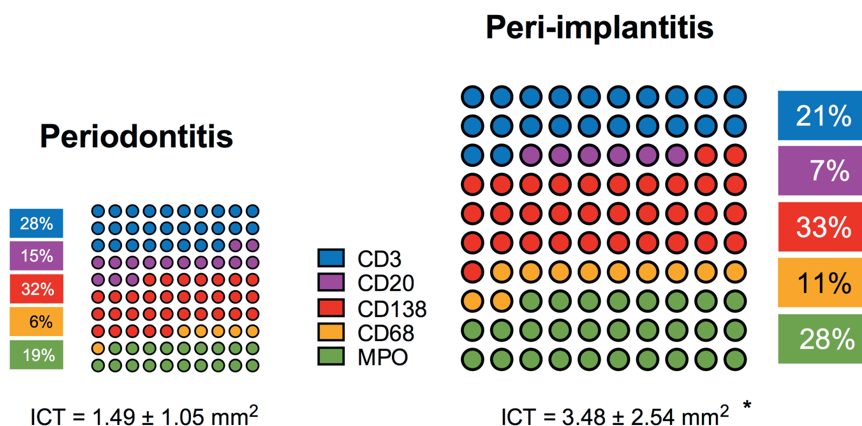
CD138-, CD68-positive cells and vascular structures (Study II)

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/mm^2 (Study II)

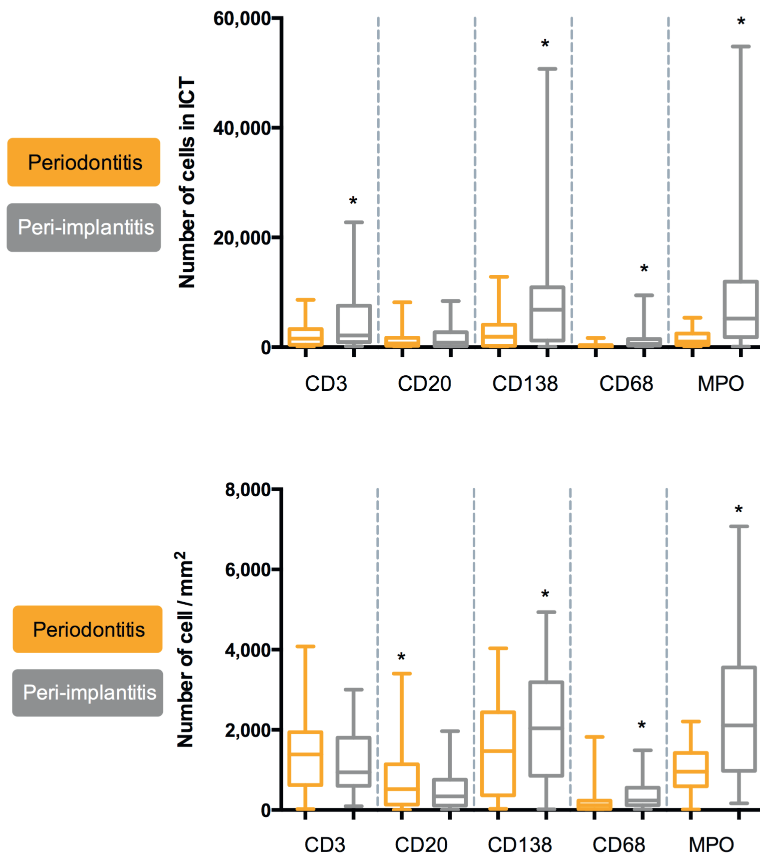
The percentage distribution of total number of cells in ICT of periodontitis and peri-implantitis lesions with the relative overall size of the ICT is presented in Figure 6. The large discrepancy on the overall size of the ICT between the 2 types of specimens is also illustrated in Figure 6.

*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 gastro-intestinal 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.

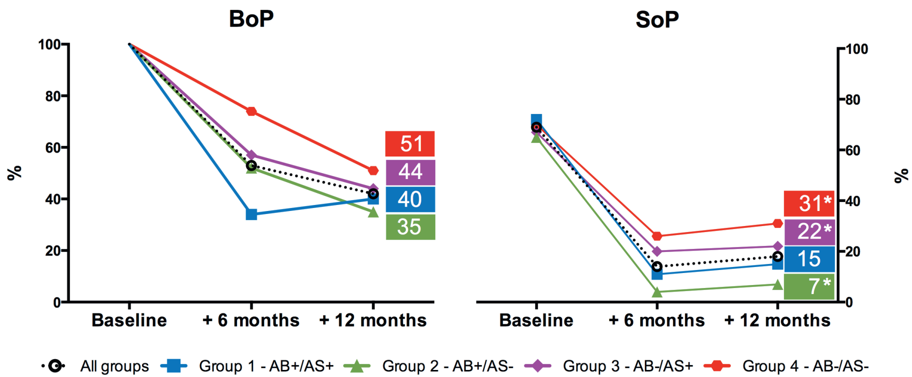
Table 13. Factors associated with treatment success: logistic regression analysis.

		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

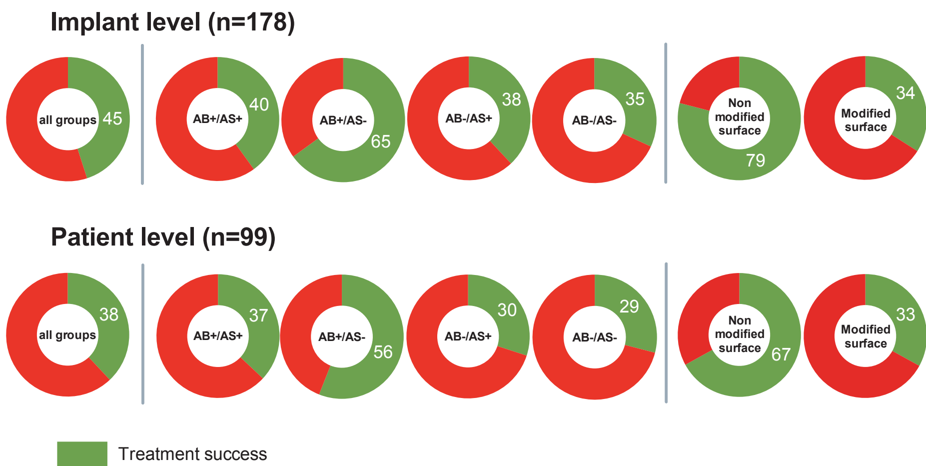
The adjunctive use of systemic antibiotics and local antiseptics had no impact on treatment success (OR 0.55; p=0.46 and OR 0.63; p=0.22 respectively), while CVD-related drug therapy had a negative effect (OR 0.21; p<0.0001).

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).

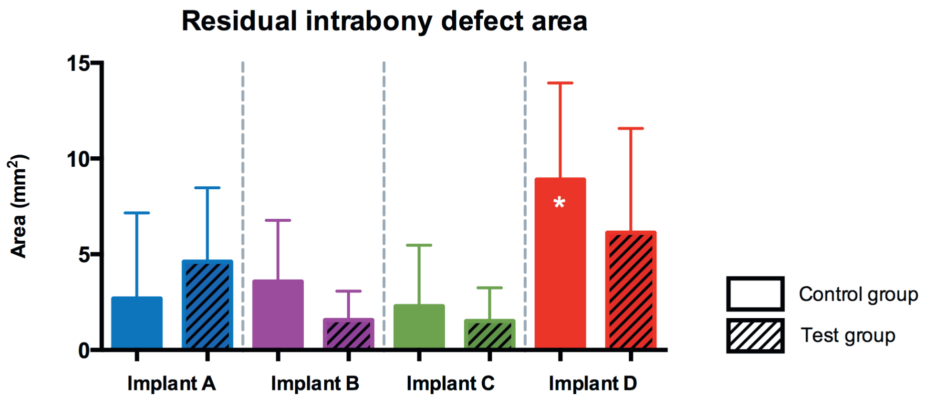
The overall distribution of the ICT scores differed between the test and control groups (Figure 11). While in implants B, C and D the test procedure resulted in lower scores than the control procedure, a reverse relationship was found for implants A. Marked differences in score distribution were also 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

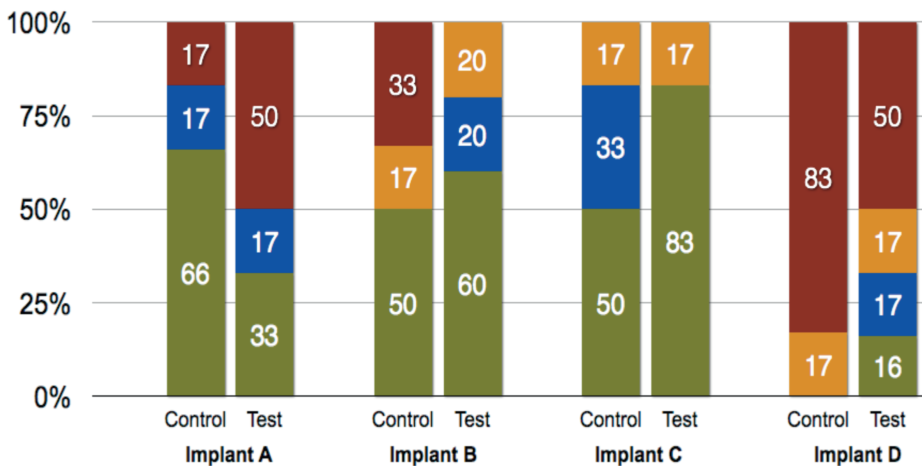
In terms of total count of bacteria, no statistically significant differences were observed among implants prior to surgery. The total count, however, had decreased significantly at 3 and 5 months after surgery in both test and control groups, except for implants D. An increase of the total DNA-probe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

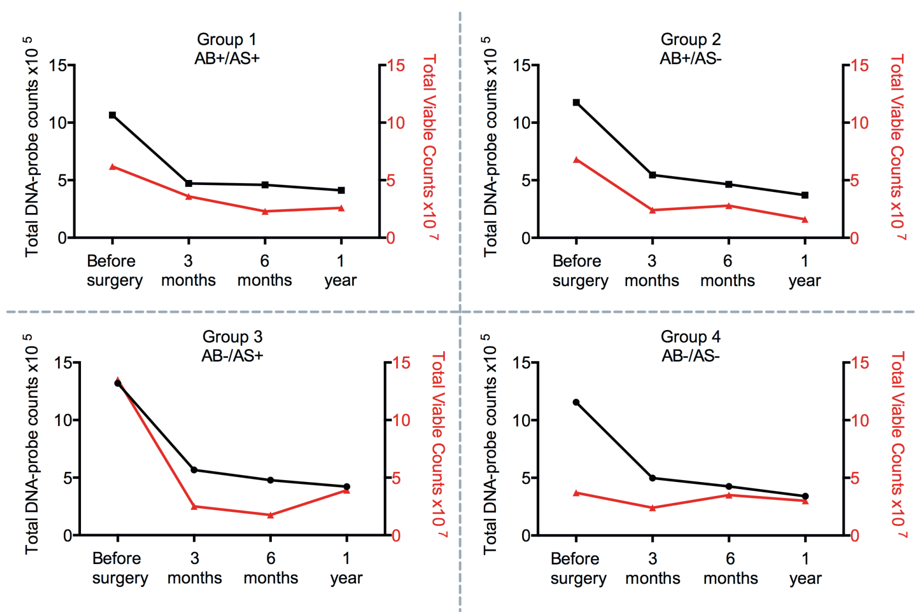


Table 15. Percentage of patients with not detected and detected bacteria (by checkerboard/culture analysis) before and 1 year after surgical treatment.

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 $\leq$ 5mm, absence of bleeding/suppuration at the 12-month examination and bone loss $\leq$ 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

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