J Clin Periodontol 2004; 31: 845–849 doi: 10.1111/j.1600-051X.2004.00567.x
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Journal of Clinical Periodontology

Spontaneous progression of experimentally induced periimplantitis

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Zitzmann NU, Berglundh T, Ericsson I, Lindhe J: Spontaneous progression of experimentally induced periimplantitis. J Clin Periodontol 2004; 31: 845–849. doi: 10.1111/j.1600-051X.2004.00567.x. © Blackwell Munksgaard, 2004.

Abstract

Background: Periimplantitis represents an inflammatory condition that is associated with the presence of a submarginal biofilm and with advanced breakdown of soft and mineralized tissues surrounding endosseous implants. Animal models have been used to describe mechanisms involved in the pathogenesis and treatment of the soft and hard tissue lesions of periimplantitis.

Objective: The aim of the present experiment was to study the presence and progression of inflammatory lesions in tissues surrounding implants exposed to ''experimental periimplantitis''.

Material and Methods: Five Labrador dogs were used. In each dog, 2 or 3 implants were placed in both the left and right edentulous premolar regions of the mandible. Abutment connection was performed 4 months later and a plaque control regimen was initiated and maintained for 5 months. ''Experimental periimplantitis'' was subsequently induced by ligature placement and plaque accumulation was allowed to progress until about 40% of the height of the supporting bone had been lost. The ligatures were removed, but plaque formation was allowed to continue for an additional 12 months. Radiographs of all implant sites were obtained before and after active ''experimental periimplantitis'' as well as at the end of the experiment. Biopsies were harvested from the implant sites in 3 of the dogs. The tissue samples were prepared for light microscopy and the sections were used for histometric and morphometric examinations.

Results: One implant was lost during the first 2 months of ''experimental periimplantitis'' and two implants were lost during the 12 months that followed ligature removal. The radiographic examination indicated that varying amounts of additional bone loss occurred in the majority of the implant sites also following ligature removal. The mucosa of all implant sites harbored inflammatory lesions that extended apically of the pocket epithelium. The lesions were separated from the marginal bone by a zone of apparently normal connective tissue.

Conclusion: A remission of the destructive inflammatory lesion in the periimplant tissues was seen in some sites following ligature removal, but in the majority of sites additional loss of supporting bone occurred.

Key words: Brånemark implants; periimplantitis

Accepted for publication 17 December 2003

Periimplantitis is defined as an inflammatory process that involves the mucosa as well as the periimplant bone and may result in advanced loss of tissue support in the affected implant site (Smith & Zarb 1989, Albrektsson & Isidor 1994). Animal models including the use of monkeys and dogs have been used to study the pathogenesis and treatment of periimplantitis (Lindhe et al. 1992, Lang et al. 1993, Schou et al. 1993, Marinello et al. 1995, Baron et al. 2000, Persson 2001). In such models, cotton ligatures were placed submarginally around implants and allowed to accumulate plaque. This resulted in inflammation and in a rapid breakdown of periimplant soft and hard tissues. Lindhe et al. (1992) reported from a study in the dog that 1 month after the removal of such ligatures, a large inflammatory cell infiltrate (ICT) resided in the periimplant mucosa (PM) and that the apical portion of the lesion

was close to or extended into the marginal bone. Marinello et al. (1995) observed that 3 months after the removal of such ligatures in ''experimental periimplantitis'', the ICT was in most sites separated from the bone by a dense collar of fibrous connective tissue. In some sites, however, the tissue destruction continued and the extensive loss of bone had caused exfoliation of the implants. The reason why, in some animals and sites, the periimplantitis lesions became encapsulated while in other sites the inflammatory condition progressed, is currently not understood.

The aim of the present experiment was to study further the condition of compromised implant sites 12 months after the removal of submarginally placed ligatures.

Material and Methods

Five Labrador dogs, about 1 year old, were used. All mandibular premolars and the first molars were extracted. After 3 months of healing, 2–3 Standard fixtures (Brånemark System®, Nobel Biocare, Göteborg, Sweden; length 10 mm, diameter 3.75 mm) were installed in the edentulous region on both sides of the mandible. A total of 22 implants were placed in the 5 dogs. Four months later, abutment connection (Standard abutments, Brånemark System®; height 4 mm) was performed.

A plaque control program was initiated. This included cleaning of teeth and implants, once a day, 5 days a week, with toothbrush and dentifrice. The plaque control regimen was terminated 5 months later, i.e. at an interval when the PM in all implant sites appeared to be clinically healthy. Cotton ligatures were placed in a submarginal position around the implants according to a method previously described (Lindhe et al. 1992). The ligatures were exchanged once every 2 weeks during a 2-month period and then removed (Period 1). At this time, approximately 40% of the initial bone support had been lost. Following ligature removal, plaque accumulation was allowed to continue for another 12 months (Period 2) after which a final examination of all implant sites was performed.

Radiographs were obtained from all implants at ligature placement, at ligature removal (2 months) and at the final examination (14 months). The radiographs were analyzed in an Olympus SZH 10 Research Stereo microscope (Olympus, Japan) and the distance between the abutment-fixture junction (A/F) and the marginal position of bone-to-implant contact (B) was determined. The measurements were made at both the mesial and the distal aspect of each implant and were redone after 1 week to confirm intraobserver reliability.

At 12 months after ligature removal, the animals were divided into two groups. Two dogs (No. 4 and 5) were assigned for treatment of periimplantitis (for details, see Persson et al. 2001), while the 3 remaining dogs (No. 1, 2, 3) were sacrificed with an overdose of Sodium-Pentothal® (Abbot laboratories, Chicago, IL, USA) and perfused through the carotid arteries with a fixative (Karnovsky 1965). The mandibles were removed and tissue samples, comprising the implant and the surrounding soft and mineralized tissues, were prepared for ground sectioning according to methods described by Donath & Breuner (1982). From each tissue block, 2 mesio-distal sections were prepared (Exakt® Apparatebau, Norderstedt, Germany) and reduced to a thickness of approximately 20 μm. The sections were stained in toluidine blue and used for histometric measurements.

Histometric analysis

The histological examinations were performed in a Leica DM-RBE® microscope (Leica, Wetzlar, Germany) equipped with an image system Q-500 MC® (Leica). In each section, the following landmarks were identified and used for linear measurements: the marginal portion of the PM, the apical termination of the barrier (pocket) epithelium (aJE), the marginal level of bone-to-implant contact (B), the most coronal level of the bone crest (BC), the abutment-fixture junction (A/F), the most apical extension of the infiltrated connective tissue (aICT), the most apical extension of the submarginal biofilm that was interposed between the implant and the pocket epithelium of the periimplant mucosa (aPlaque).

The distances between the landmarks were measured in a direction parallel with the long axis of the implant, while the width of the bone defect (BC-implant) was assessed in a direction perpendicular to the implant. The measurements were performed at the mesial and the distal aspects of each implant.

The size of the inflammatory cell infiltrate (area ICT) in the mucosa was assessed by outlining the entire circumference of the lesion with a mouse cursor. The surface area occupied by plaque and calculus (area plaque) and by pus (area pus) adjacent to the implant was determined in a similar manner. Mean values were calculated for each implant site, dog and examination interval.

Results

Clinical observations

During Period 1 (ligature breakdown; 0–2 months) one implant was lost (Dog 1). Two additional implants (Dog 2) were lost during Period 2 (2–14 months). The clinical examinations performed at 2 and 14 months revealed that all remaining implants harbored large amounts of plaque and calculus. Further, the PM of all sites exhibited marked signs of inflammation.

Radiographic observations (5 animals)

The mean amount of bone loss that occurred during Period 1 (0–2 months) was 2.58 ± 0.39 mm (Table 1). During Period 2 additional bone loss occurred (1.02 ± 1.62 mm). This additional bone loss varied considerably between animals and implants (Fig. 1). Thus in 5 implants, there was in Period 2 no obvious bone loss, while moderate bone loss (0–1 mm) was observed in 10 implant sites. In 2 implants, the additional amount of bone loss varied between 1 and 2 mm, and in 4 implants, there was pronounced further loss of bone, i.e. exceeding 2 mm (Fig. 1). In this group of 4 implant units, the 2 implants were included which were lost prior to the 14-month examination.

Histological observations (3 animals)

The PM at all implant sites examined, harbored a large inflammatory cell infiltrate (Figs 2a,b). An ulcerated pocket epithelium separated the infiltrated connective tissue from plaque and pus in the pocket area. Calculus was occasionally found on the implant surface.

The inflammatory cell infiltrate (ICT) contained large numbers of plasma cells, lymphocytes, neutrophilic leukocytes (PMN-cells) and macrophages. Further, the ICT area (1) included a large number of vascular structures, (2) was almost devoid of collagen and fibroblasts, and

Table 1. Results from the radiographic examination A/F-B, mean SD

Dog	Ligature placement	Ligature removal (2 months)	End of study (14 months)
1	1.94 (0.51)	4.27 (0.62)	5.32 (1.92)
2	1.95 (0.85)	4.23 (0.97)	8.04 (2.96)*
3	2.06 (0.58)	4.46 (0.48)	4.59 (1.80)
4	1.73 (0.37)	4.41 (0.23)	4.34 (0.98)
5	1.32 (0.31)	4.55 (0.43)	4.70 (0.80)
Mean values	1.80 (0.29)	4.38 (0.13)	5.40 (1.52)
	Period 1 (0–2 months) active lig. breakdown	Period 2 (2–14 months) spontaneous progression	
Bone loss that took place during Period 1 and Period 2 ($n = 5$)	2.58 (0.39)	1.02 (1.62)	

*Including 2 implants lost during Period 2.

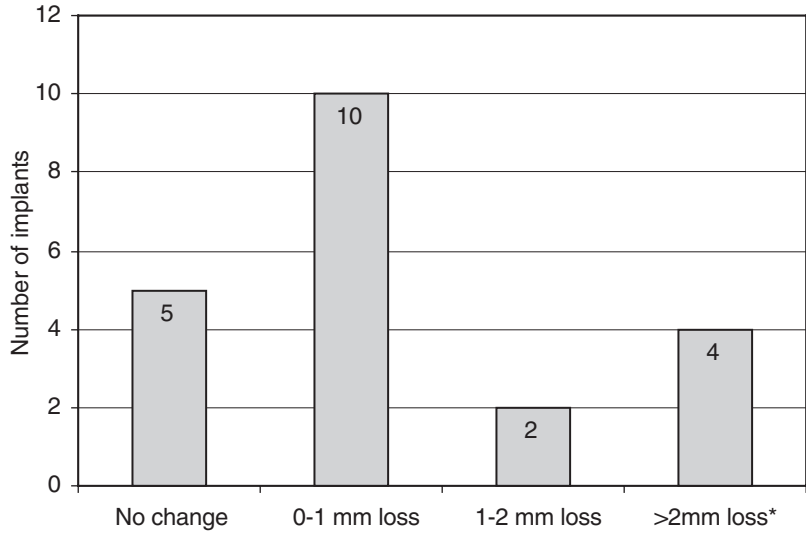


Fig. 1. Frequency distribution of implants with varying amount of radiographic bone loss during Period 2 (2–14 months).

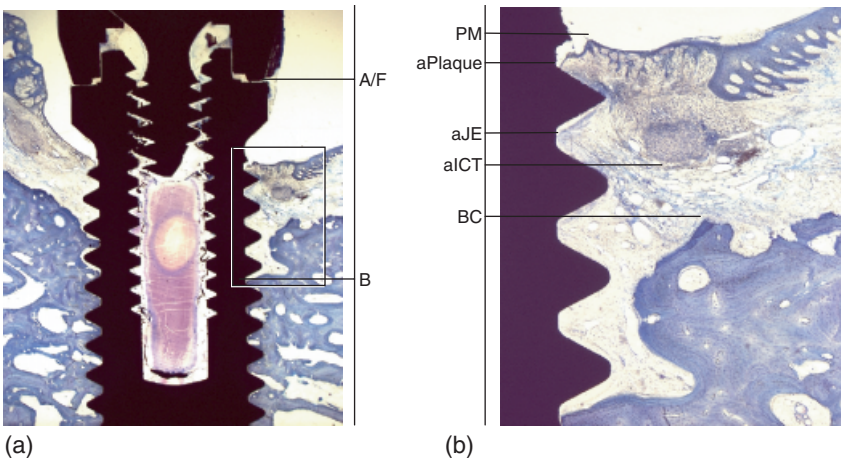


Fig. 2. (a) Mesio-distal cross section of the periimplant tissues adjacent to the implant (overview). PAS and toluidine blue, original magnification × 16. (b) Higher magnification (× 50) of the marginal portion of the mesial aspect. A/F, PM, aPlaque, aJE, aICT, B and BC indicate the landmarks used for histometric measurements.

(3) was in an apical direction separated from bone tissue by a dense connective tissue (Figs 3a, b). This connective tissue contained large amounts of fibroblasts and collagen but no or only scattered inflammatory cells (Figs 4a,b). Within the adjacent marginal bone tissue, secondary osteons and reversal lines were frequently present while osteoclasts were found only occasionally.

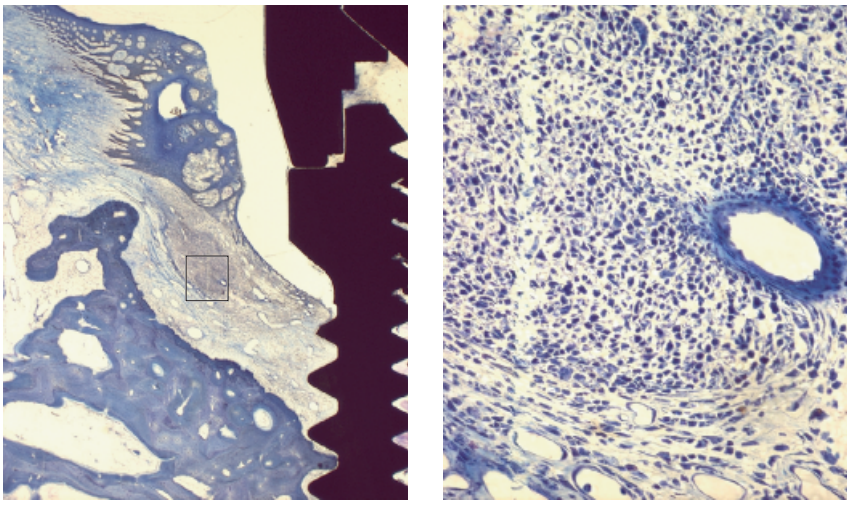
Histometric findings

The results from the histometric measurements are reported in Table 2. The mean height of the periimplant mucosa (PM-B) was 5.66 ± 1.24 mm and the length of the barrier epithelium (PM-aJE) was on the average 1.35 ± 0.58 mm. The connective tissue located between the epithelium and the bone (aJE-B) had a mean height of 4.31 ± 1.61 mm. The ICT extended a distance of 4.47 ± 1.60 mm apical of the mucosal margin (PM-aICT). The mean distance between the abutment-fixture junction and the marginal level of the bone-to-implant contact (A/F-B) was 5.66 ± 0.73 mm. The vertical dimension of the bone defect (B-BC) was on the average 4.10 ± 0.94 mm, while the corresponding horizontal dimension (BC-imp) measured 2.55 ± 0.49 mm.

The mean size of the ICT was $2.64 \pm 1.05\,mm^2$, and areas occupied by plaque and by pus were on the average 0.69 ± 0.77 and $0.79 \pm 0.72\,mm^2$, respectively.

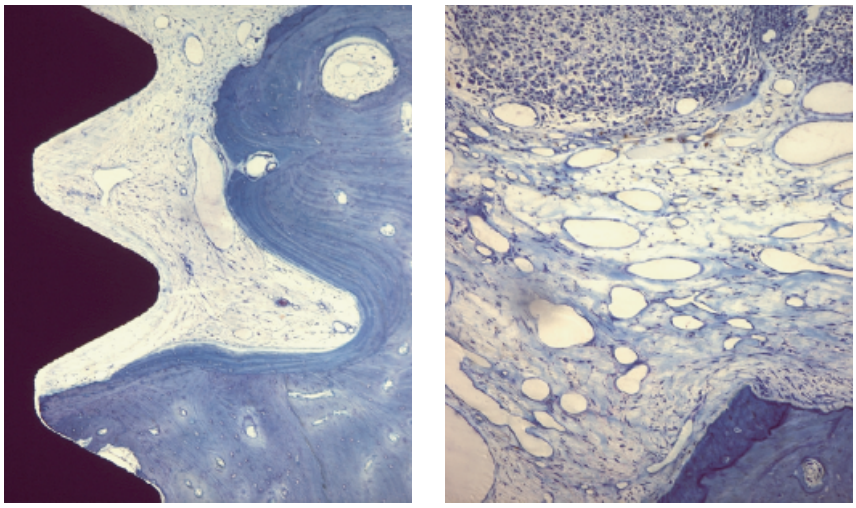
Discussion

In the present study on ''experimental periimplantitis'' the marginal bone levels at 21 implants in 5 dogs were monitored in radiographs: (i) at ligature placement, (ii) immediately after ligature removal at 2 months, and (iii) after a subsequent 12-month period of continued plaque accumulation (14 months). The findings demonstrated that in this model 16 out of 21 implants exhibited varying amounts of additional bone loss during the 12-month (Period 2) that followed ligature removal. Further, the histological examination of biopsies from 3 dogs obtained at the end of the experiment (14 months) revealed that the mucosa of all sites harbored large inflammatory lesions that extended apically of the pocket epithelium, and that this inflammatory cell infiltrate was consistently separated from the periimplant bone by a zone of



(a) (b)

Fig. 3. (a) Marginal portion of the distal aspect of the implant illustrated in Fig. 2a (× 25). Note pocket epithelium, ICT and a wide, crater-like bone defect. (b) Higher magnification (× 400) of the ICT area.



(a) (b)

Fig. 4. (a) Higher magnification (× 100) of the apical area of Fig. 2b. (b) Note the non-inflammatory zone interposed between the bone and the ICT (× 200).

Table 2. Results from the histometric measurements, dimensions in mm (SD)

Dog	1	2	3	mean (SD)
PM-B	5.85	6.79	4.33	5.66 (1.24)
PM-aJE	0.72	1.45	1.87	1.35 (0.58)
aJE-B	5.13	5.34	2.46	4.31 (1.61)
A/F-B	5.56	6.43	4.99	5.66 (0.73)
B-BC (defect depth)	4.22	4.98	3.11	4.10 (0.94)
BC-imp (defect width)	1.99	2.89	2.76	2.55 (0.49)
PM-aICT	3.81	6.30	3.31	4.47 (1.60)
area ICT (mm^2)	2.02	3.85	2.04	2.64 (1.05)
aPlaque-B	2.13	1.75	2.13	2.00 (0.22)
area plaque (mm^2)	0.13	1.57	0.37	0.69 (0.77)
area pus (mm^2)	0.28	1.61	0.49	0.79 (0.72)

apparently non-inflamed (non-infiltrated) connective tissue.

The finding that ligature placement and plaque formation (''experimental periimplantitis'') at an implant site resulted in soft tissue inflammation and rapid bone loss is in agreement with data previously reported (e.g. Lindhe et al. 1992, Marinello et al. 1995, Persson et al. 2001). Thus, in the interval between 0 and 2 months about 2.5–3 mm of the marginal bone was lost. It is suggested that the mechanical separation of the attachment between the mucosa and the implant, and the buildup of plaque in this submarginal location initiated an inflammatory reaction that involved not only the mucosa but also the periimplant bone.

The observation that the progressive breakdown of the periimplant bone during Period 2 continued in some but not all implant sites, is in agreement with findings reported by Marinello et al. (1995). They induced ''experimental periimplantitis'' in Labrador dogs and examined the soft and hard tissue lesions following 1 and 3 months after ligature removal. It was stated that in one of the dogs followed for 3 months, ''3 of the 4 implants were lost between the 10th and 12th week examination interval. In the remaining dogs, all implants were found to be present and stable.'' (Marinello et al. 1995). The reason why some periimplantitis lesions are associated with extensive and others with minor bone loss is currently not understood, but may be related to differences between implant sites regarding the submarginal microbiota, or the quality of the host's response to the infection.

The histological examination of the current biopsy material showed that at the end of Period 2, a zone of normal connective tissue consistently separated the inflammatory lesion from the marginal bone. Further, no or only isolated osteoclasts could be found on the surface of the marginal bone. In other words, at the time of biopsy, there were no signs of ongoing bone resorption. In this context, it should be realized that during the 12 months of monitoring during Period 2, the animals continued to accumulate plaque and calculus. The present findings are thus, in agreement with the data presented by Marinello et al. (1995). In their sections, representing 1 month following ligature removal, the inflammatory lesion extended from the mucosa into the marrow spaces of the supporting bone.

On the contrary, in sections representing 3 months after ligature removal, most of the lesions were encapsulated and separated from the bone by a collagen-rich connective tissue (Marinello et al. 1995).

Ethical statement

The protocol of the present study was approved by the Regional Ethics Committee for Animal Research, Sweden.

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