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Impact of implant overloading on the peri-implant bone in inflamed and non-inflamed peri-implant mucosa

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Abstract

Objective: To assess the impact of overloading on peri-implant bone level and the bone-to-implant contact (BIC) in the presence of healthy or inflamed peri-implant tissues.

Materials and methods: Four screw-shaped machined implants were placed bilaterally in the mandible of four beagle dogs and left submerged for 3 months. Prosthetic abutments were connected either in supra-occlusal contact with the opposite teeth (overloaded) or in infra-occlusal position (unloaded). In each dog, cotton floss ligatures were placed unilaterally around abutments to promote plaque accumulation; the contralateral side was brushed three times a week. There were four experimental sites, two implants in each: loaded uninflamed (LU), loaded inflamed (LI), unloaded uninflamed (UU), and unloaded inflamed (UI). Clinical and radiographic parameters were recorded at baseline and every 3 months throughout the observation period. At 12 months, the dogs were sacrificed and histomorphometric analysis was performed.

Results: Implants with ligature-induced peri-implantitis presented high inflammatory indices throughout the observation period. Clinical parameters did not change from baseline for both LU and UU. Loading significantly increased the percentage of BIC (BIC%) ($P<0.05$) and slightly increased crestal bone resorption, but not apical to the implant neck. Both LI and UI groups showed significant peri-implant bone loss ($P<0.01$), mostly horizontal on the buccal aspect and angular on the lingual aspect, which exposed implant threads. Loading significantly ($P<0.05$) increased implant thread exposure due to buccal and lingual vertical bone resorption.

Conclusions: In the presence of uninflamed peri-implant mucosa, overloading of implants in the dog model increased BIC% and slightly reduced marginal bone level. However, resorption did not progress beyond the implant neck. Overloading aggravated the plaque-induced bone resorption when peri-implant inflammation was present.

Optimal oral hygiene and proper occlusion are considered critical for long-term success of endosseous oral implants (van Steenberghe et al. 1999; Kim et al. 2005). Clinical studies show a correlation between improper oral hygiene and inflammation of the peri-implant mucosa, which can lead to peri-implant marginal bone resorption (Lindquist et al. 1988, 1996; Becker et al. 1990; Sanz et al. 1991; Schou et al. 1992; Salonen et al. 1993; Leimola-Virtanen et al. 1995; Teixeira et al. 1997). The effect of plaque on the peri-implant tissues has been assessed in human and animal studies and shows an inflammatory response with numerous common features with the reaction of gingival tissue to plaque accumulated on the tooth surfaces (Berglundh et al.

1992; Lindhe et al. 1992; Lang et al. 1993; Schou et al. 1993; Pontoniero et al. 1994; Hanisch et al. 1997).

In clinical reports and reviews, overloading has been suggested as an additional major factor that could cause marginal peri-implant bone loss that would eventually result in implant failure (Adell et al. 1981; Cox & Zarb 1987; Lindquist et al. 1988; Jemt et al. 1989; Quirynen & Listgarten 1990; Rosenberg et al. 1991; Sanz et al. 1991; Naert et al. 1992; Quirynen et al. 1992; Ranger et al. 1995; Esposito et al. 1997; van Steenberghe et al. 1999; Kim et al. 2005).

Animal studies have provided evidence of marginal bone loss associated with excessive occlusal and repetitive loading in the absence of peri-implant tissue inflammation (Hoshaw et al. 1994; Isidor 1996, 1997; Miyata et al. 2000). Conversely, other experimental studies using various animal models have shown that occlusal stress does not cause peri-implant bone loss in the absence of peri-implant infection (Ogiso et al. 1994; Barbier & Schepers 1997; Miyata et al. 1998; Gotfredsen et al. 2001a, 2001b, 2001c; Heitz-Mayfield et al. 2004), or when combined with peri-implant soft tissue inflammation (Hürzeler et al. 1998; Gotfredsen et al. 2002). There are still insufficient data in the literature and no conclusive evidence regarding the contribution of excessive occlusal load in the pathogenesis of peri-implant marginal bone loss.

The objective of the present study was to assess the impact of overloading on osseointegrated implants with machined surface in the presence or absence of experimentally induced peri-implant inflammation.

Materials and methods

The study protocol was approved by the Ethics and Institutional Animal Care and Use Committees of Tel-Aviv University. The experiment was conducted on four 2-year-old beagle dogs, weighing 13.5–16 kg. Animals were housed individually and maintained on a commercial diet and water *ad libitum*. All surgical procedures were performed under general anesthesia, using intravenous sodium pentobarbital (25 mg/kg). Dogs were administered with 2 cc of xylazine base and Benzanthine Penicillin G (2% chanazine) (Chanelle Pharmaceuticals Manufacturing Ltd., Longhrea Co, Galway, UK) at 20 mg/kg as pre-medication. Local infiltration of Lidocaine with norepinephrine (1 : 100,000) was administered for hemostasis and reduction of post-operative pain.

After periodontal examination and scaling, all four mandibular premolars (PM 1–4) were extracted bilaterally. At 3 months, a full-thickness flap was elevated and four internal hexed, screw-type machined titanium implants (Hi-Tec Implant Ltd., Herzliya, Israel), 3.75 × 10 mm with a 2.7 mm implant neck, were inserted into the edentulous area on each side, following the Brånemark two-stage surgical protocol (Adell et al. 1987). Implants were placed with their coronal margin at the level of the alveolar bone crest (Fig. 1). Flaps were sutured over the cover screws. After 3 months of healing, all 32 implants were exposed and 3-mm healing screws were connected. Post-operatively, surgical sites were swabbed three times a week with 0.2% chlorhexidine digluconate (Corsodyl®, GlaxoSmithKline, München, Germany). At 3 weeks healing screws were removed. In each quadrant, prosthetic abutments, 5 and 8 mm long, were connected to the two anterior and two posterior implants, respectively. The 5-mm-long abutments were free of any occlusal contact (i.e., unloaded). However, a definitive tight premature supra-occlusal contact was created between each 8 mm long abutment and a flat occlusal plane prepared from microhybrid posterior composite material (Filtek™ P-60 3M ESPE, St Paul, MN, USA) on the opposite teeth (Fig. 2). The excessive height of the implant suprastructure resulted in disarticulation and an increased anterior vertical dimension of 3–4 mm. Each time the animal occluded, the implants and opposite teeth were subjected to increased vertical load, i.e., overloaded due to a dynamic force that exceeded that of the normal physiologic range as described previously by Budtz-Jogensen (1980) in an experimental model designed to induce bruxism and trauma from occlusion. The excessive load applied to the implants was in an axial rather than lateral direction and was not quantified directly, but assessed by evaluating the influence of the overload on the periodontium of the opposite teeth.

Fig. 1. Four implants placed with their coronal margin coinciding with the level of bone crest.

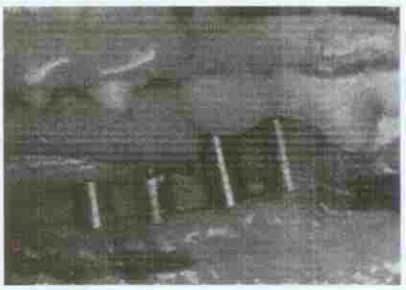

Fig. 2. Two unloaded implants free of occlusal contact and two overloaded implants in which a supra-occlusal contact with the opposite teeth provides axial loads to the implants. This picture is taken from the uninflamed group as reflected by plaque free abutment surfaces and healthy peri-implant mucosa.

Consequently, on each side of the mandible, each dog received two overloaded implants in supra-occlusal contact in the distal area of the mandible (area of P3 and P4) and two anterior-unloaded implants (area of P1 and P2). On one side, cotton floss ligatures were placed around the abutments to promote plaque accumulation and to induce plaque-associated peri-implant inflammation (Lang et al. 1993; Shibli et al. 2003). Ligatures were secured apically to the prominence of the abutment, maintaining contact with the peri-implant mucosal margins. During the experiment, ligatures were replaced every 4 weeks and secured in a submarginal position close to the level of abutment prominence. On the contralateral side, abutments were meticulously brushed with 0.2% chlorhexidine digluconate solution (Corsodyl®) 3 times a week. In each dog, there were four experimental sites, each containing two implants: unloaded uninflamed (UU), loaded uninflamed (LU), unloaded inflamed (UI), and loaded inflamed (LI).

Before placing the ligatures, measurements were taken at baseline for the

peri-implant bone level (using periapical radiographs), implant stability (using the Periotest system (Periotest, Siemens AG, Bensheim, Germany) (Isidor 1998), manual mobility of opposite teeth, clinical measurements of the peri-implant mucosa consisting of a modified Plaque Index (mPI), (Mombelli et al. 1987), modified Gingival Index (mGI) (Lobene et al. 1986), peri-implant probing (PPD), and probing of the opposite teeth (PPD) at six sites using a round millimeter-graded plastic manual probe (Vivacare TPS Probe (universal explorer), Vivadent ets, Schaan, Liechtenstein) with a standardized pressure of 0.20 N. Clinical measurements and Periotest recordings were repeated monthly and radiographs were taken every 3 months. The presence of a supra-occlusal contact of the loaded implants was checked monthly and, if needed, composite material was added to the occlusal surface of the opposite teeth to maintain the increased vertical dimension. Dogs were sacrificed 12 months after ligature placement by an intravenous lethal overdose of sodium pentobarbital injection. A solution of 10% neutral-buffered formalin was injected into the external carotid artery (Karnovsky 1965) to achieve tissue fixation before specimen block removal. Mandible tissue blocks were separated, immersed in 10% formalin, and radiographed before histologic processing.

Histologic preparation and histomorphometry

Specimens were dehydrated with a graded series of ethanol for 9 days and infiltrated with a light-polymerized embedding resin (Technovit 7200 VLC, Heraeus Kulzer GmbH Co, KG, Hanau, Germany). After 20 days of infiltration with constant shaking at a normal atmospheric pressure, specimens were embedded and polymerized by 450 nm light at 40 °C. Sections were prepared using the cutting/grinding method described by Donath & Breuner (1982) applying the EXAKT cutting/grinding system (EXAKT Technologies, Oklahoma City, OK, USA). Tissue blocks were cut into 150-μm-thick vertical bucco-lingual sections in the long axis of the implants. All sections were polished to a thickness of 40 μm using the EXAKT microgrinding system, followed by alumina polishing paste, and stained with Stevenel's blue. Sections taken from the center of the implant were viewed in a Zeiss Axioplan 2 microscope (Carl Zeiss Microimaging Inc., Thornwood, NJ, USA) and digital images were obtained by an Optronics DEI-750 camera (Optronics, Goleta, CA, USA). These images were used for morphometric measurements with the Bioquant Nova Image Analysis System (Bioquant Image Analysis Corp, Nashville, TN, USA).

Two types of measurements were taken: linear distances between defined points along the implant (e.g., top of the implant, which is also the top of the implant neck, bottom of the implant neck, which is close to the first

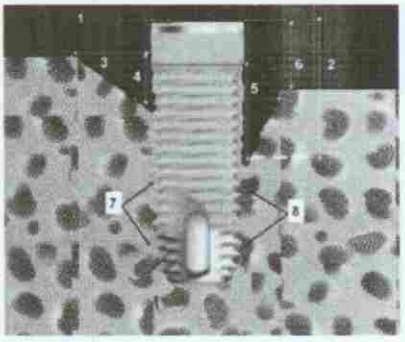

Fig. 3. Scheme illustrating the landmarks of the implants and the histomorphometric parameters measured. – (red) bottom of implant neck. (1) Original bone level at the day of implant placement. (2) tNe/BI, distance between the top of the implant neck to the initial bone contact. (3) IBH, intrabony defect; horizontal distance. (4) IBV, intrabony defect; vertical distance. (5) bNe/BI, distance between the bottom of implant neck to initial bone contact. (6) tNe/CrB, distance between the top of the implant neck to crestal bone. (7) BIC, linear bone-implant contact surface. (8) MIC, linear marrow-implant contact surface.

Table 1. **Average of morphometric measurements (mm) in the different experimental groups* (mean ± standard deviation)**

Parameter	Uninflamed		Inflamed		Two-way ANOVA		
	Unloaded (UU)	Loaded (LU)	Unloaded (UI)	Loaded (LI)	Inflammation	Loading	Inflammation/loading interaction
BIC	12.68 ± 3.11[a]	16.38 ± 1.85[a]	9.74 ± 1.93	9.31 ± 1.46	$P=0.05$		$P=0.03$
MIC	11.09 ± 1.6	8.72 ± 2.68	5.69 ± 1.8	4.72 ± 2.19	$P=0.008$		
TIH	23.76 ± 2.08	25.10 ± 1.05	15.43 ± 3.47	14.03 ± 3.34	$P=0.009$		
BIC (%)	53 ± 9.48[b]	66.01 ± 9.19[b]	62.68 ± 4.32	67.53 ± 9.37	$P=0.045$		
tNe/BI-B	2.61 ± 0.74	2.54 ± 0.58	5.49 ± 1.08	5.91 ± 0.89	$P=0.016$		
tNe/BI-L	1.98 ± 0.68	2.23 ± 0.65	5.46 ± 1.11	6.08 ± 0.33	$P=0.008$		
IBV-B	1.32 ± 0.88	0.69 ± 0.67	1.01 ± 0.37	1.83 ± 0.83			
IBH-B	0.39 ± 0.23	0.41 ± 0.34	1.20 ± 0.35	1.69 ± 0.72			
IBV-L	1.54 ± 0.9	1.03 ± 0.39	4.14 ± 1.92	4.85 ± 0.83	$P=0.034$		
IBH-L	0.72 ± 0.57	0.53 ± 0.19	2.20 ± 0.07	2.35 ± 0.33	$P=0.003$		
Ne/BI-B†	0.37 ± 0.54	0.12 ± 0.61	−2.53 ± 0.77[c]	−3.08 ± 0.89[c]	$P=0.015$	$P=0.05$	
bNe/BI-L†	0.8 ± 0.68	0.50 ± 0.64	−2.50 ± 0.65[d]	−3.28 ± 0.31[d]	$P=0.006$	$P=0.016$	
tNe/CrB-B	1.28 ± 0.33	1.85 ± 0.22	4.47 ± 0.83	4.08 ± 0.23	$P=0.001$		
tNe/CrB-L	0.43 ± 0.37	1.20 ± 0.44	1.31 ± 0.95	1.23 ± 0.57			

*$N=4$ per group.

†Positive and negative values indicate a bone level coronal and apical (−), respectively to bottom of implant neck.

Values with [similar superscript letters] are statistically different (Paired *t*-test $P<0.05$).

BIC, bone-implant contact (length); STC, soft tissue-implant contact (length); TIH, total implant osseous housing (BIC + STC); BIC%, percentage of bone as percent of the total osseous housing; tNe/BI, distance between the top of the implant neck to the initial bone-to-implant contact on buccal tNe/BI-B and lingual tNe/BI-L aspect; IBV, intrabony defect vertical dimension on buccal IBV-B and lingual IBV-L; IBH, intrabony defect horizontal dimension on buccal IBH-B and lingual IBH-L; bNe/BI, distance between the bottom of implant neck to initial bone to implant contact on buccal bNe/BI-B and lingual bNe/BI-L; tNe/CrB, distance between the top of implant neck to crestal bone level on buccal tNe/Cr-B and lingual tNe/Cr-L.

implant thread) and reference points at the peri-implant bone (e.g., crestal bone level, coronal level of bone-to-implant (BIC) contact), and surfaces, by tracing the implant perimeter in contact with bone or marrow.

The following parameters were measured (Fig. 3):

- BIC – length of direct contact between bone-to-implant surface.
- MIC – length of contact between bone marrow to the implant surface.
- TIH – total implant housing (= BIC + MIC).
- BIC% – bone-implant contact as percent of the total osseous housing (= BIC × 100/TIH).
- tNe/BI – distance between the top of the implant neck to the initial bone-to-implant contact (i.e., defect bottom) on the buccal (tNe/BI-B) and lingual (tNe/BI-L) aspects.
- tNe/CrB – distance between the top of the implant neck to the crestal bone level on the buccal (tNe/CrB-B) and lingual (tNe/CrB-L) aspects.
- bNe/BI – distance between the bottom of the implant neck to the initial bone-to-implant contact on the buccal (bNe/BI-B) and lingual (bNe/BI-L) aspects.
- IBV – vertical dimension of the intrabony defect on the buccal (IBV-B) and lingual (IBV-L) aspects.
- IBH – horizontal dimension of the intrabony defect (between the crestal bone level and the implant body) on the buccal (IBH-B) and lingual (IBH-L) aspects.

Histomorphometric parameters were divided into those that measure horizontal bone loss (tNe/CrB), vertical bone loss (IBV, IBH), total bone loss along the implant surface (tNe/BI, bNe/BI), and parameters related to the remaining bony support (BIC, BIC%, MIC, TIH).

Statistical analysis

As each dog had two implants (repeats) in each experimental site, data were first analyzed with analysis of variance (ANOVA) with repeated measures where inflammation, loading, and repeat were the within-subject factors. The effects of the repeat were not significant for any of the variables; thus, measurements from each similar pair of sites were averaged. Subsequently, ANOVA with repeated measures was re-applied with inflammation and load as the within-subject factors. In most of the variables, only inflammation had a significant effect (see Table 1). In the few variables where both inflammation and load had significant effects or interaction, paired *t*-tests were used to further analyze the data.

Results

Clinical parameters

At the beginning of the study, dogs presented slight gingivitis but no alveolar bone loss. At second-stage surgery, all implants were stable and healed uneventfully (mean Periotest value −1.58). At baseline, the mean pocket depth was 1.99 ± 0.48 mm, mean mPI 0.19 ± 0.087, and mean mGI 0.085 ± 0.078. At 1 month, the mean mPI and mGI significantly increased in the inflamed sites regardless of loading (1.94 ± 0.19 and 1.99 ± 0.21, respectively), consistent with progressive peri-implant inflammation. During the study, excellent plaque control and healthy peri-implant mucosa were observed in the LU and UU sites (Fig. 2). By the end of the experiment, the mean mPI and mGI further increased to 2.85 ± 0.12 and 2.94 ± 0.08 (LI), and 2.90 ± 0.15 and 2.95 ± 0.1 (UI), respectively. Also, the mean peri-implant pocket depth significantly increased compared with baseline (7.16 ± 1.14 mm and 7.28 ± 0.9 mm in UI and LI, respectively). During the study, all implants were stable with a mean Periotest value of −0.76 and a non-significant difference between the various groups. Over the 12-month study, minor wear of the composite material covering the occlusal tooth plane opposite the implant suprastructure was observed. Teeth opposite the implant superstructure presented manual mobility, moderate attachment loss, and radiographic evidence of marginal and inter-radicular alveolar bone loss and intrusion (Fig. 4a). At the end of 12 months, radiographs revealed marked peri-implant bone loss, extending onto the implant threads in the inflamed sites and crestal bone changes confined to the implant neck in the uninflamed sites (Fig. 4b and c).

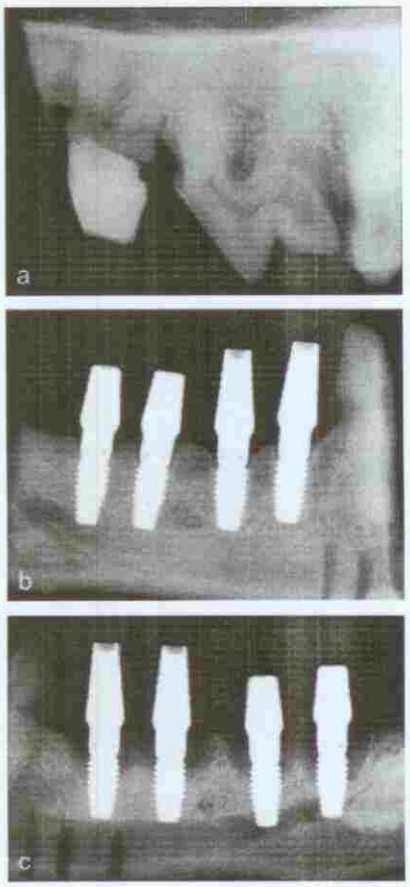

Fig. 4. Radiographs taken at the end of the experiment. (a) Opposite tooth (P4) presenting radiographic evidence of marginal and inter-radicular alveolar bone loss and intrusion. (b) Uninflamed site: slight crestal bone resorption apically to the top of the implant neck but not beyond the bottom of the neck. (c) Inflamed site: pronounced circumferential bone resorption with significant implant thread exposure in both overloaded and unloaded implants.

Histomorphometric results

Effect of inflammation

Implants with ligature-induced peri-implantitis showed severe bone loss on both lingual and buccal aspects (Table 1, Fig. 5a and b). All parameters with quantitated total (horizontal + vertical) bone loss were significantly affected (Table 1). In the unloaded implants, the mean distance between the top of the implant neck to the initial BIC (tNe/BI) on the buccal and lingual aspects increased from 2.61 ± 0.74 and 1.98 ± 0.68 mm, respectively, in the uninflamed (UU) sites to 5.49 ± 1.08 and 5.46 ± 1.11 mm, respectively, in the inflamed (UI) sites. Similar changes were noted in overloaded implants from 2.54 ± 0.58 and 2.23 ± 0.65 mm, respectively, in uninflamed (LU) sites to 5.91 ± 0.89 and 6.08 ± 0.33 mm in the

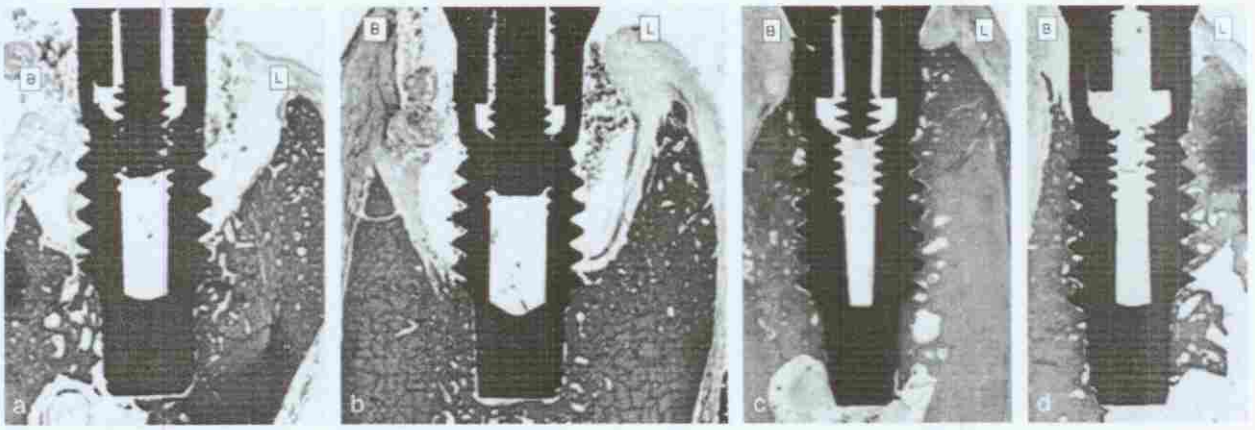

Fig. 5. Bucco-lingual cross-section of experimental sites (original magnification × 10). (a) Unloaded inflamed site: extensive peri-implant bone resorption approximately up to the apical one-third of the implant body. Bone loss is mostly horizontal with slight vertical resorption on the buccal aspect and mostly vertical on the lingual aspect of the implant. (b) Loaded inflamed site: extensive peri-implant vertical bone resorption on both aspects of the implant. (c) Unloaded uninflamed site: the bone crest is located on the implant neck. (d) Loaded uninflamed site: marginal bone located on the implant neck with shallow intrabony bone defect configuration extending close to the implant threads.

inflamed (LI) ones. These changes were well reflected in the height of the initial BIC relative to the bottom of the neck (bNe/BI). In implants surrounded by uninflamed soft tissue, this parameter on the buccal and lingual aspects had a positive value, indicating that, on average, the bottom of the bony defect in uninflamed sites contacted the implant neck coronally to the implant threads. In contrast, bNe/BI (buccal and lingual) in the inflamed sites had a negative value, which indicated that the bottom of the defect contacted the implant threads.

In the inflamed sites, horizontal and vertical bone loss was observed on the buccal and lingual aspects. Horizontal bone resorption was prominent on the buccal aspect, as reflected by a significant ($P<0.005$) increase in the distance between the top of the implant neck and crestal bone level (Table 1; tNe/CrB), from 1.28 ± 0.33 mm (UU) to 4.47 ± 0.83 mm (UI); from 1.85 ± 0.22 mm (LU) to 4.08 ± 0.23 mm (LI). Horizontal bone resorption on the lingual aspect was much less pronounced, from 0.43 ± 0.37 and 1.20 ± 0.44 mm (UU and LU implants, respectively), to 1.31 ± 0.95 and 1.23 ± 0.57 mm (UI and LI, respectively). However, inflammation greatly increased the intrabony vertical distance on the lingual aspect (Table 1: IBVL) from 1.54 ± 0.9 mm (UU) to 4.14 ± 1.92 mm (UI), which indicated a significant ($P<0.05$) inflammation-related vertical bone loss. In contrast, vertical resorption was much less pronounced on the buccal aspect.

Inflammation also increased the horizontal dimension of the intrabony defect (Table 1: IBH) from 0.39 ± 0.23 mm (UU) to 1.2 ± 0.35 mm (UI) on the buccal aspect, and from 0.72 ± 0.57 mm (UU) to 2.2 ± 0.07 mm (UI) on the lingual aspect ($P<0.005$).

As inflammation caused significant bone loss, BIC and the total implant osseous housing (TIH) were greatly reduced, from 12.68 ± 3.11 and 23.76 ± 2.08 mm (UU) to 9.74 ± 1.93 and 15.43 ± 3.47 mm (UI), respectively. However, inflammation did not change the BIC%.

Effect of overloading

Uninflamed sites, regardless of loading, displayed bone resorption along the implant neck. The initial BIC in these sites was located 1.98–2.61 mm apical to the top of the implant (Table 1: tNe/BI), and 0.1–0.8 mm coronally to the bottom of the implant neck and the first thread. Overloading did not affect the marginal bone level. However, overloading slightly increased the horizontal bone loss as measured by the distance between the top of the implant and crestal bone level (Table 1: tNe/CrB), which increased from 1.28 ± 0.6 mm (UU) to 1.85 ± 0.39 mm (LU) on the buccal aspect and from 0.43 ± 0.45 mm (UU) to 1.2 ± 0.67 mm (LU) on the lingual aspect. This effect was not statistically significant and was much smaller than that of inflammation.

In the absence of inflammation, overloading as compared with unloaded implants significantly ($P<0.05$) increased both BIC, from 12.68 ± 3.11 mm (UU) to 16.38 ± 1.85 mm (LU), and BIC% from 53 ± 9.48 (UU) to 66.01 ± 9.19 (LU), (Table 1, Fig. 5c and d). In contrast, overloading had no effect on either BIC or BIC%, and on the magnitude of horizontal bone resorption as measured by the distance between the top of the implant neck to crestal bone level (tNe/CrB) in implants surrounded by *inflamed* soft tissue. However, overloading enhanced peri-implant intrabony vertical bone resorption, which resulted in significant ($P<0.05$) increased bone loss along the buccal and lingual aspects of the implant threads (measured by the distance between the bottom of the implant neck and the initial BIC (Table 1: bNe/BI).

Discussion

In the present study, a dog animal model was used to investigate the relationship between overloading of implants surrounded by uninflamed or inflamed soft tissue on the one hand, and peri-implant bone level and bone-to-implant bone contact on the other. Experimental chronic mucositis and peri-implantitis in the dog are considered to be a valuable model to

evaluate the etiology, progression, and treatment of peri-implant diseases. Comparability between the magnitude of vertical bite force in humans and dogs shows similarity (Bunski et al. 2000) enabling the use of a dog model to assess dynamic overloading of dental implants. There is no precise definition of 'overload' and no accepted physically quantified amounts of load or overload (Hoshaw et al. 1994). Instead, an undesirable pathologic response of the mineralized tissues at the interface of the applied load supports the determination of the applied load at certain biological milieu as 'overload.' As the loading regimen used in the present study resulted in distinct signs of trauma from occlusion in the opposite teeth (increased mobility, radiographic evidence of breakdown of marginal and inter-radicular alveolar bone, and intrusion), the applied load can reliably be defined as 'overload.'

The present study indicated that in a healthy peri-implant environment, overloading by itself did not induce pocket formation. In spite of the micromotion related to occlusal overload, which can disrupt the fragile contact between the implant and epithelium or connective tissue and increases the risk of bacterial plaque penetration (Miyata et al. 2002), clinical attachment loss did not occur during the 12-month experimental period. This phenomenon is comparable with the lack of pathologic reaction of the healthy dentogingival unit, which does not react to occlusal trauma by pocket formation (Svanberg & Lindhe 1973; Polson et al. 1976).

In both loaded and unloaded implants with healthy peri-implant soft tissue, histomorphometric measurements presented crestal (0.43–1.28 mm) and marginal (1.98–2.61 mm) bone loss along the machined non-screw-shaped implant neck, which did not extend apically to the bottom of the implant neck. This reduction in peri-implant bone level may be related to surgical trauma (van Steenberghe et al. 1999), establishment of a biological width apically to the implant neck-abutment connection at the expense of crestal bone, and the microgap phenomenon (Berglundh & Lindhe 1996; Weber et al. 1996; Cochran et al. 1997; Hermann et al. 1997, 2000; Vogel et al. 1999). Furthermore, as the machined implant neck was initially placed at the bone level, the observed bone resorption could have been an outcome of the remodeling process caused by the fibrous encapsulation of the bone at the interface of the implant neck (Thomas & Cook 1985; Hämmerle et al. 1996), or lack of effective mechanical coupling between the bone and the machined implant surface, which could lead to bone loss due to disuse atrophy (Al-Sayyed et al. 1994).

In healthy peri-implant experimental sites, overloading slightly increased crestal horizontal bone loss, compared with unloaded implants, but not beyond the level of the bottom of the implant neck. The non screw-shaped configured implant neck, unlike the thread configuration, does not protect the bone from the stress and strain of the applied dynamic overload (Van Oosterwyck et al. 1998). Thus, the bone loss may be related to the micro-damage in the bone tissue (Frost 1994; Hoshaw et al. 1994; Duyck et al. 2001) initiated by stress concentration and stress shielding at the implant neck (Bidez & Misch 1992; Kitamura et al. 2004; Misch et al. 2004). Recently, it has been demonstrated in a dog model that normal functional loads, as applied at implants by connecting a fixed partial denture with 'occlusal contacts with appropriate load distribution,' do not trigger a specific load-induced bone response and thus do not result in marginal bone loss (Berglundh et al. 2005).

The present results confirmed other animal studies that have not been able to demonstrate significant peri-implant bone loss following occlusal overloading in the absence of inflammation (Ogiso et al. 1994; Barbier & Schepers 1997; Miyata et al. 1998; Gotfredsen et al. 2001a, 2001b, 2001c; Heitz-Mayfield et al. 2004). However, there are some contradicting reports (Hoshaw et al. 1994; Isidor 1996, 1997; Miyata et al. 2000).

The present study used a ligature-induced peri-implant infection animal model originally developed in a germ-free and conventional rat model (Rovin et al. 1966) to induce a rapid periodontal tissue lesion. The ligatures placed in germ-free rats induced only a slight gingival inflammation, whereas an intense inflammatory response was seen in the conventional animals. This finding suggests that mechanical irritation *per se* is of little importance in causing gingival inflammation. Ligature placement around implants allows subgingival microbiota to be established and enhances bacterial accumulation, followed by pocket formation and rapid peri-implant bone loss (Hickey et al. 1991; Leonhardt et al. 1992; Lindhe et al. 1992; Schou et al. 1993; Shibli et al. 2003). Therefore, the peri-implantitis lesion occurs as the result of plaque accumulation and not because of mechanical trauma from the ligature or as an acute foreign-body reaction (Lindhe et al. 1992; Mombelli & Lang 1994). In the present study similar to other studies mucositis initiated by plaque accumulation was followed by development of peri-implantitis (Berglundh et al. 1991; Ericsson et al. 1992; Lindhe et al. 1992; Lang et al. 1993; Tillmanns et al. 1997, 1998; Shibli et al. 2003; Martins et al. 2004, 2005). Ligature removal may lead to unpredictable results, either encapsulation of the lesion that converts the active, destructive inflammatory lesion to a 'resting' lesion (Marinello et al. 1995) with large variations of the lesion's size and extension (Lindhe et al. 1992; Gotfredsen et al. 2002), or to further progression (Marinello et al. 1995; Martins et al. 2005). Therefore, in order to prevent variability in progression of the chronic peri-implantitis lesion due to ligature removal, we kept the ligature in permanent contact with the marginal peri-implant tissue throughout the experiment.

Histomorphometric measurements revealed that the plaque-induced peri-implant inflammation caused significant bone resorption along the implant surface and exposed the implant threads. The association between plaque accumulation and crestal bone resorption has been demonstrated in human (Adell et al. 1981; Lindquist et al. 1988; Becker et al. 1990; Rosenberg et al. 1991; Sanz et al. 1991) and animal (Lindhe et al. 1992; Lang et al. 1993) studies. In the present study, horizontal and angular bone resorption participated in the deterioration of peri-implant support in both overloaded and unloaded implants. Horizontal bone resorption was primarily on the buccal aspect, and the angular type of resorption on the lingual aspect of the implant in both groups. This could be explained by the differences in marginal bone width on both implant aspects. The buccal plate of the osteotomy site in the experimental animals had a