Microbiology and Cytokine Levels Around Healthy Dental Implants and Teeth

Hessam Nowzari, DDS, PhD;* Javier Enrique Botero, DDS, PhD;† Marina DeGiacomo, MD;‡ Maria C. Villacres, PhD;§ Sandra K. Rich, RDH, PhD‖

ABSTRACT

Background: Elicitation of the relationship of periodontopathogens and pro-inflammatory cytokines to bone resorption and formation is significant to a growing body of research known as osteoimmunology. It is essential that clinically healthy peri-implant and periodontal sites are studied to contribute comparison data for investigations that are addressing diseased sites.

Purpose: The purpose of this study was to describe levels of selected pro-inflammatory cytokines in clinically healthy peri-implant and periodontal sites, and to examine whether cytokine levels may be related to specific bacterial/viral pathogens.

Materials and Methods: Eleven subjects (mean age 56.2 ± 10) participated in the study. Subgingival microbial samples were cultured for periodontopathic bacteria. Gingival crevicular fluid samples were analyzed by nested polymerase chain reaction for Cytomegalovirus (HCMV) and were tested for the quantification of Interleukin (IL)-8, IL-1β, IL-6, IL-10, Tumor Necrosis Factor (TNF)-α, and IL-12p70 using flow cytometry (FACS). Findings for microbiota composition and cytokine levels were compared between implants and teeth (chi square, Kruskall–Wallis, Mann–Whitney; $p \leq .05$).

Results: Both the frequency (%) and levels (%) of periodontopathic bacteria were higher around teeth than implants. The concentration (picogram per milliliter) of cytokines was more prominent around implants than teeth, reaching nearly twofold differences in some instances. Cytokine levels were higher when the sites analyzed were positive for any bacteria tested. HCMV was not detected.

Conclusions: Pro-inflammatory cytokine production was unrelated to heavy bacterial challenge. Nevertheless, when periodontopathic bacteria were detected by culture, cytokine levels were increased around both implants and teeth. Studies are needed to investigate the pro-inflammatory cytokines (especially IL-1β and TNF-α) produced in spite of minimal bacterial accumulation.

KEY WORDS: cytokines, Cytomegalovirus, healthy implants, oral immunology, subgingival microbiota

INTRODUCTION

Periodontal and peri-implant bone turnover is a balanced dynamic process that involves resorption and formation, controlled and influenced by the local production of cytokines, with a wide range of inflammatory, hemopoietic, metabolic, and immunomodulatory properties.[1,2]

Peri-implant microbial contamination or infection (bacteria and viruses) elicits an immune response regulated by key cytokines (TNF-α, interleukin [IL]-1β, Transforming Growth Factor (TGF)-β, IL-10) that control the progression and/or suppression of the inflammatory response. Overproduction of pro-inflammatory cytokines released by monocytes/ macrophages and T cells in response to microbial challenge

*Professor, clinical dentistry, and director, advanced periodontics, University of Southern California School of Dentistry, Los Angeles, CA, USA; †assistant professor, Periodontal Medicine Group, School of Dentistry, Universidad del Valle, Cali-Colombia; ‡researcher, Department of Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; §assistant professor of research, Department of Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; ‖associate professor, advanced periodontics, University of Southern California School of Dentistry, Los Angeles, CA, USA

Reprint requests: Dr. Hessam Nowzari, University of Southern California Dental Science Center-DEN, 925 W. 34th Street, Room 119, Los Angeles, CA 90089-0641, USA; e-mail: nowzari@usc.edu

DOI 10.1111/j.1708-8208.2007.00076.x

can lead to the breakdown of the periodontal or peri-implant tissues.[3] It has been observed that the subgingival microbiota around implants affected by pocketing and bone loss presented high levels of periodontal pathogens, and periodontally involved teeth in partially edentulous patients may serve as microbial reservoirs.[4,5] In addition, surgical trauma in part is responsible for an early hyper-inflammatory response, which is characterized by both TNF-α and IL-1β release.[6] On the other hand, ions released from dental implants can stimulate peripheral blood mononuclear cells (PBMCs) to produce IL-1β and TNF-α in vitro.[7] Commercially pure titanium and titanium alloys also have been associated with the production of other cytokines such as IL-6 and IL-18.[8]

IL-1β and TNF-α appear to play major roles in mediating the inflammatory response in the pathogenesis of many chronic inflammatory diseases such as rheumatoid arthritis.[9,10] IL-1β is present at elevated levels in the gingival crevicular fluid (GCF) in the course of periodontitis and peri-implant inflammation.[11,12] It is produced primarily by monocytes, but may be produced by other nucleated cells in response to injury.[7] TNF-α, a cytokine with some functions similar to those of IL-1β, has been detected in sites affected by periodontitis.[13] Moreover, TNF-α and IL-1β act synergistically to initiate the cascade of inflammatory mediators.[14] IL-6 has pro-inflammatory effects and is responsible for collagen resorption of gingival tissues,[15] while IL-10 is an inhibitor of inflammation.[16] Other cytokines, such as IL-12, appear to induce the secretion of Interferon (IFN)-γ from activated T and Natural Killer (NK) cells,[17] and IL-8 acts as a potent chemoattractant for neuthrophils[18] in gingival tissues. Nonetheless, the impact of cytokine levels on healthy implants has not been studied.

The complex and active network of cytokines and their receptors around clinically healthy dental implants should be further investigated to assess the interaction of cytokines, bacteria, and viral variables on peri-implant bone and soft tissue. Therefore, the purpose of this study was to describe levels of selected pro-inflammatory cytokines in clinically healthy peri-implant and periodontal sites, and to examine whether cytokine levels may be related to specific bacterial/viral pathogens.

MATERIALS AND METHODS

Patients of the advanced periodontics clinic at the University of Southern California were invited to participate in the study. The study design and protocol were approved by the University of Southern California, Health Sciences Institutional Review Board.

Subjects and Clinical Examination

Demographic and general information regarding the type of implant, dimensions, and date of placement were collected for each implant. Implants included in the study were considered clinically healthy in that no pocketing (≥4 mm), no bleeding on probing (BOP), and no bone loss beyond the first thread were detected. In order to compare implants to teeth, one tooth was selected in each patient. The teeth were also free from any signs of destructive periodontal disease (ie, periodontitis: pocket formation, BOP, and bone loss).

Patients were excluded who (1) had periodontal and/or implant disease, (2) were pregnant or lactating, or (3) had antibiotic intake 3 months before inclusion.

All examinations/samplings of any patient were performed by the same clinician.

Clinical parameters were recorded for each implant/tooth studied at the mesial, buccal, distal, and lingual sites using a marked periodontal probe (CP12, Hu-Friedy, Chicago, IL, USA) as follows: mean probing depth in millimeter (PD) and mean BOP as positive/negative sites. The clinical parameters were recorded immediately after GFC and microbial sampling to avoid alterations induced by probing.

GFC Sampling and Cytokine Analysis

GFC was collected using periopaper strips (Periopaper, Oraflow, Plainview, NY, USA).

Strips were gently placed in the mesial and distal sites for 1 minute and then transferred to a centrifuge tube containing 100 μL of phosphate buffered saline (PBS). Strips contaminated with blood were discarded. During sampling, the tubes were maintained in dry ice and immediately frozen at −70°C until processing.

For analysis, samples were incubated at 4°C for 12 hours and then vortexed at maximum speed for 20 minutes. Inflammatory cytokines were quantified using a cytometric bead array (BD Biosciences, San Diego, CA, USA). The samples and positive controls (standard curve) were processed according to the manufacturer instructions, and the values for IL-8, IL-1β, IL-6, IL-10, TNF-α, and IL-12p70 were calculated and presented as picogram per milliliter. Data were acquired in a FACS-

Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

Subgingival Microbiological Analysis

Bacterial Examination. Supragingival plaque was carefully removed using a sterile gauze before sampling around implants and teeth. Three sterile paper points (Johnson & Johnson, East Windsor, NY, USA) were inserted to the bottom of the sulcus and left in place for 30 seconds, and then transferred to a vial containing Viability Medium Göteborg Anaerobically (VMGA) III transport medium.[19] The same procedure was repeated, and the paper points were placed in a centrifuge tube and frozen immediately (–70°C) for polymerase chain reaction (PCR) analysis. Samples were immediately delivered for processing at the Oral Microbiology Testing Laboratory of the University of Southern California. The samples were processed within 2 hours of collection. Anaerobic microbiological isolation and identification of putative periodontal pathogens were carried out with no knowledge of the source of the specimens, following established procedures. The samples were dispersed on a Vortex mixer (Fisher Scientific, Pittsburgh, PA, USA) at the maximal setting for 45 seconds, and then 10-fold serially diluted in VMG I anaerobic dispersion solution. Using a sterile bent glass rod, 0.1-mL aliquots from 103 to 105 dilutions were plated onto nonselective 4.3% brucella agar (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with 0.3% bactoagar, 5% defibrinated sheep blood, 0.2% hemolyzed sheep red blood cells, 0.0005% hemin, and 0.00005% menadione. Total viable counts and proportions of specific bacteria in relationship to the total viable counts were determined. Aliquots diluted in VMGA III medium were plated onto tryptic soy-serum-bacitracin-vancomycin (TSBV) medium for the culture of *Actinobacillus actinomycetemcomitans*, enteric Gram-negative rods and yeasts.[20] The nonselective blood agar was incubated at 35°C in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI, USA) containing 85% N2–10% H2–5% CO2 for 10 days. TSBV medium was incubated in 10% CO2 in air at 37°C for 4 days. Presumptive identification of representative colonies of each group of organism that morphologically resembled the study species was performed according to the methods described by Slots[21] and by the use of a micromethod system (API 20°, bioMérieux, Marcy l'Etoile, France). Organisms examined included *A. actinomycetemcomitans*, *Prevotella Intermedia, Prevotella nigrescens*, *Porphyromonas gingivalis*, *Dialister pneumosintes*, *Tannerella forsythia*, *Campylobacter* spp., *Fusobacterium* spp., *Micromonas micros*, enteric Gram-negative rods, and *Candida* spp.

Bacteria designated as major periodontal pathogens included *A. actinomycetemcomitans*, *P. gingivalis*, *D. pneumosintes*, and *T. forsythia*. The percentage recovery of periodontal pathogens was determined by the colony count of each microbial taxon in relation to the total viable count. The specific patient information was not included with the samples to ensure blinding of the laboratory personnel performing the microbiological testings.

Viral Examination. DNA extraction and nested PCR for the detection of HCMV was carried out according to Parra and Slots[22] and Contreras and Slots.[23] Briefly, 500 μL of TE buffer (10 mM Tris-hydrochloride, 1 mM EDTA, pH 7.5) was added to the microcentrifuge vial containing the paper points and was vortexed for 10 minutes. Nucleic acid was bound to silica particles in the presence of guanidinium thiocynate (GuSCN) lysis buffer. Nucleic acid/silica was recovered by centrifugation (12,000 *g* for 30 seconds), washed in buffer (GuSCN-Tris-hydrochloride), twice in 70% ethanol and once in acetone, and then dried in a heating block at 56°C for 10 minutes. The nucleic acid/silica pellet was resuspended in 100 μL TE buffer, incubated at 56°C for 10 minutes, and centrifuged (12,000 *g* for 2 minutes), and the supernatant was stored at –70°C.

Nested PCR was conducted to detect cytomegalovirus (HCMV), and amplification products (136 bp) were electrophoresed on 2% agarose gels, stained with ethidium bromide (0.5 μg/mL), and observed under UV light (300 nm) transilluminator. Data are presented as the frequency detection (%) of HCMV positive subjects.

Statistical Analysis

Demographic, clinical, and immunological information was collected and analyzed using a statistical software (GraphPad Prism version 4.00 for Windows, San Diego, CA, USA). Data are presented as mean, standard deviation, and standard error of the mean. The frequency detection of microorganisms and HCMV was analyzed using the chi-square test. The levels of cultured bacteria were analyzed with the Kruskall–Wallis test.

TABLE 1 Demographic and Clinical Description of Study Sample

Parameter	Subjects	
Number	11	
Age, mean ± SD	56.2 ± 10	
Gender	Female, 10/Male, 1	
	Implants	**Teeth**
Number	12	11
Implant type (*n*)	Nobel Biocare SG (1)	
	3i Osseotite XP (5)	
	3i Osseotite NT (2)	
	3i Osseotite certain (1)	
	3i Osseotite (2)	
	3i Osseotite micromini (1)	
Bleeding on probing, *n* (%)	2/48 (4.1%)	1/44 (2.2%)
Probing depth, mean ± SEM	2.1 ± 0.1	1.58 ± 0.1

Nobel Biocare SG, Nobel Biocare USA, LLC, 22715 Savi Ranch Parkway, Yorba Linda, CA 92887, USA.
3i Osseotite XP, 3i Osseotite NT, 3i Osseotite certain, 3i Osseotite, and 3i Osseotite micromini, BIOMET 3i, 4555 Riverside Drive, Palm Beach Gardens, FL 33410, USA.
SEM = standard error of the mean.

Cytokine concentration was assessed using the Mann–Whitney test. Statistical significance was assumed when $p \leq .05$.

RESULTS

Eleven subjects, mean age 56.2 years old, presenting one or more implants were included in the study. In general, one implant and one tooth were studied in every subject, except in one patient where two implants were studied. In total, 12 implants and 11 teeth were analyzed clinically, microbiologically, and immunologically. The mean BOP and PD were slightly higher around implants than teeth, but the difference was not statistically significant (Table 1). Although a minimum BOP was observed in isolated sites (4.1% [2/48] implants, 2.2% [1/44] teeth), implants and teeth were considered clinically healthy. BOP could have been induced by probing. For this reason, the clinical parameters were recorded after subgingival microbial and GCF sampling.

Table 2 shows the frequency of detection (%) and levels (%) of putative periodontopathic bacteria in subgingival plaque samples around implants and teeth. Higher frequency and higher levels of periodontopathic bacteria were detected around teeth. *P. gingivalis*, *T. forsythia*, *Fusobacterium* spp., and enteric rods were the most representative. *P. intermedia* was elevated around implants. Overall, more periodontopathic bacteria were detected around the teeth, but the difference was not statistically significant. Human Cytomegalovirus (HCMV) was not detected in any of the samples analyzed around implants and teeth (Table 2).

Levels of cytokines in GCF were analyzed using flow cytometry. First, we analyzed for differences in the levels (picogram per milliliter) of inflammatory cytokines between implants and teeth (Table 3). The concentration of cytokines was more prominent around implants than teeth, reaching almost a twofold difference in some instances. That was the case for TNF-α (6.57 pg/mL vs 2.06 pg/mL) and IL-8 (4,285 pg/mL vs 1,592 pg/mL) in GCF from implants and teeth, respectively ($p < .01$).

Second, we studied if the presence of at least one microorganism as detected by culture had an impact on the levels of inflammatory cytokines (Table 4). In general, the levels (picogram per milliliter) of TNF-α, IL-10, IL-1β, and IL-8 were increased both around implants and teeth sites positive for any of the microorganisms tested. More specifically, TNF-α and IL-10 were increased and statistically significant around implants ($p < .05$). In contrast, TNF-α, IL-1β, and IL-8 were elevated in concentration around the teeth, but the difference was not statistically significant. IL-1β had a

TABLE 2 Frequency Detection (%) and Levels (%) of Subgingival Pathogens/Human Cytomegalovirus (HCMV) Around Implants and Teeth

	Implants		Teeth	
Microorganisms	Frequency (%)	Levels (%)	Frequency (%)	Levels (%)
Actinobacillus actinomycetemcomitans	0	0	0	0
Porphyromonas gingivalis	16.7	0.7	27.3	1.3
Prevotella intermedia	16.7	0.9	9.1	0.7
Tannerella forsythia	25	1.3	27.3	1.5
Campylobacter spp.	25	1.4	27.3	1.5
Eubacterium spp.	8.3	0.3	18.2	1.1
Fusobacterium spp.	16.7	1.0	36.4	1.7
Micromonas micros	0	0	9.1	0.4
Enteric rods	16.7	1.6	27.3	2.6
Eikenella corrodens	0	0	0	0
Dialister pneumosintes	0	0	0	0
HCMV	0	NA	0	NA

$p \leq .05$. No significant differences were observed.
Chi square was used to test for differences in the frequency (%) detection of periodontal pathogens and HCMV. Kruskall–Wallis test assessed the levels (%) of periodontal pathogens.
NA = not applicable.

twofold increase in levels in culture-positive sites around implants and teeth.

DISCUSSION

The continuous balance that exists between the host immune response and potential subgingival pathogens (bacteria/viruses) determines the clinical condition, not only around the teeth, but also around osseointegrated dental implants. In this investigation, we analyzed the production of cytokines around clinically healthy teeth and dental implants, and examined their relationship to putative periodontal pathogens. We first determined the microbial composition of subgingival plaque using culture around teeth and implants (Table 2), and although no specific microbiological profile was observed, the teeth allowed for more colonization by *P. gingivalis*, *T. forsythia*, and Fusobacterium spp. Microscopic structural differences between dental and implant surfaces could account for this finding.

There is no information available on the detection of HCMV around dental implants. In contrast to implants, HCMV has been detected in low frequencies around periodontally healthy teeth. The samples analyzed in this study were negative for HCMV as studied by nested PCR. The absence of prominent inflammation could help explain this result. Studies addressing a potential pathologic role of HCMV around implants are needed.

Inflammatory cytokines are present in low levels in clinically healthy periodontal tissues.[15] We asked if there was any difference in cytokine production between teeth and implants (Table 3). A tendency to a more cytokine production was observed around implants in contrast to teeth, but a specific explanation for this finding is not available. It can be implied that an implant acts as a foreign object and results in cytokine secretion. This

TABLE 3 Cytokine Levels (pg/mL) in Gingival Crevicular Fluid Around Implants and Teeth

Cytokine	Implants pg/mL ± SEM	Teeth pg/mL ± SEM	*p* value
IL-12	0.25 ± 0.17	0.12 ± 0.12	NT
TNF-α	6.57 ± 1.26	2.06 ± 0.53	0.006*
IL-10	11.8 ± 1.75	8.3 ± 2.46	0.074
IL-6	68.3 ± 46.41	12.32 ± 3.99	0.085
IL-1β	767.4 ± 176.1	515.6 ± 81.57	0.538
IL-8	4,285 ± 807.9	1,592 ± 200.2	0.003*

*Two-tailed Mann–Whitney test was used to analyze data ($p \leq 0.05$).
SEM = standard error of the mean; NT = not tested.

TABLE 4 Cytokine Levels (pg/mL) in Gingival Crevicular Fluid Around Implants and Teeth According to Microbial Detection

	Implants			Teeth		
Cytokine	Culture positive pg/mL ± SEM	Culture negative pg/mL ± SEM	*p* value	Culture positive pg/mL ± SEM	Culture negative pg/mL ± SEM	*p* value*
IL-12	0.3 ± 0.3	0.2 ± 0.2	NT	0	0.3 ± 0.2	NT
TNF-α	10.4 ± 2.1	4.7 ± 1.1	0.027*	2.5 ± 0.8	1.6 ± 0.71	0.580
IL-10	16.8 ± 1.9	9.3 ± 2.0	0.042*	9.6 ± 4.4	6.8 ± 1.8	0.855
IL-6	26.9 ± 12.1	89.1 ± 69.7	1.00	7.5 ± 2.0	18.2 ± 8.12	0.522
IL-1β	1,190.7 ± 364.7	555.7 ± 159.3	0.062	639.2 ± 97.5	367.3 ± 110.7	0.144
IL-8	4,929.8 ± 1,960.0	3,962.2 ± 818.2	0.865	1,777.2 ± 281.2	1,368.7 ± 281.0	0.465

*Two-tailed Mann–Whitney test was used to analyze data ($p \leq .05$).
Culture positive samples were defined as at least one of the 11 microorganisms tested by culture.
SEM = standard error of the mean; NT = not tested.

raises the issue of an immune response against the chemical components of the implant. Perala and colleagues[24] indicated that dental implant surfaces may lead to an activation of human PBMCs for the secretion of IL-1β and TNF-α.

Titanium particles in vitro have been shown to influence the release of IL-2, TNF-α, and IL-6.[25] Sedarat and colleagues[26], in an in vitro controlled experiment, exposed titanium implants to an environment similar to in vivo conditions and measured 16 (+ or −5) ng/cm^2/day dissolution of titanium and titanium alloy over a 96-day period. The dissolution of titanium/titanium alloy and the ions released by the atomic process of biodegradation can explain, at least in part, the presence of cytokines where no microbial pathogens could be detected. The other contents of commercially pure titanium implants, such as carbon, iron, nitrogen, oxygen, and hydrogen, require further evaluations.

In the present study, the production of cytokines in healthy sites may be representative of an inevitable and continuous microbial challenge around teeth and implants. We then asked if the presence of specific periodontal pathogens had an impact on cytokine production around teeth and implants (Table 4). We consistently found that subjects who were positive for at least one of the 11 microorganisms tested by culture had higher levels of IL-1β, TNF-α, IL-10, and IL-8 at teeth and implant sites. Virulence factors from periodontopathic bacteria (eg, *P. gingivalis*) are potent stimulants for the secretion of pro-inflammatory cytokines (IL-1β and TNF-α) and the subsequent activation of matrix metalloproteinases (MMP 2) and other collagenases from gingival fibroblasts.[27] Taking into consideration that active IL-1β and TNF-α mediate a variety of biological functions including osteoclast activation,[28] leukocyte recruitment, and excessive production of MMPs,[29] the overproduction of these cytokines at some time point could lead to bone resorption and collagen degradation. In addition, the production of IL-8 in gingival tissues is an important mechanism of polymorphonuclear neutrophils (PMNs) recruitment and constitutes a first line of immune defense. PMNs produce IL-1β in response to bacterial challenge and act in a paracrine way preventing apoptosis and increasing the phagocytic activity of other PMNs.[30] Low counts of PMNs in clinically healthy gingival tissues are a common finding in histological analysis at teeth and implant sites.[31] The balance between this innate response and the bacterial challenge is partly responsible for maintaining the health of gingival tissues. Nevertheless, although previous studies have reported that cytokine activity seems to be relevant for alveolar bone resorption and destruction of collagen,[32,33] periodontal research, to date, has not yet established any particular cytokine profile that could be of predictive value for disease progression. Moreover, there is no known cytokine level threshold that could differentiate between a stable site and the initiation of a pathologic process in periodontal and peri-implant tissues.

Both the design and composition of dental implants must be considered as possible contributing factors to immune response, especially when important microbial challenge and inflammation is not evidenced. Our data provide a basis for further developing and testing

hypotheses related to cytokine profiles, implant designs, and peri-implant bone and soft tissue height. In this study, regardless of the absence of a major bacterial challenge, cytokine levels of IL-1β and TNF-α were substantial at peri-implant sites. These cytokines are potent stimulators of bone resorption and may be involved in the inflammatory response and early bone loss after dental implant placement.

CONCLUSIONS

Important periodontopathic bacteria were detected in relatively low frequency and levels around clinically healthy implants and teeth. Cytokine production was higher around implants than teeth, indicating that factors other than bacterial plaque accumulation may account for this difference. Nevertheless, when periodontopathic bacteria were detected by culture, cytokine levels were increased both around implants and teeth.

Studies are needed to investigate the pro-inflammatory cytokines (especially IL-1β and TNF-α) produced in spite of minimal bacterial accumulation.

ACKNOWLEDGMENTS

This study was supported by the University of Southern California Advanced Periodontics Program research funds. Javier Enrique Botero holds a scholarship from, and would like to thank, COLCIENCIAS (Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología Francisco José de Caldas).

REFERENCES

1. Arai K, Lee F, Miyajima A, Miyatake S, Arai N, Yokota T. Cytokines: coordinators of immune and inflammatory responses. Annu Rev Biochem 1990; 59:783–836.
2. Callard R, George AJ, Stark J. Cytokines, chaos and complexity. Immunity 1999; 11:507–513.
3. Baker PJ, Garneau J, Howe L, Roopenian DC. T-cell contributions to alveolar bone loss in response to oral infection with *Prophyromonas gingivalis*. Acta Odontol Scand 2001; 59:222–225.
4. Hultin M, Gustafsson A, Hallström H, LÅ J, Ekfeldt A, Klinge B. Microbiological findings and host response in patients with peri-implantitis. Clin Oral Impl Res 2002; 13:349–358.
5. Botero JE, Gonzalez AM, Mercado RA, Olave G, Contreras A. Subgingival microbiota in peri-implant mucosa lesions and adjacent teeth in partially edentulous patients. J Periodontol 2005; 76:1490–1495.
6. Menger MD, Vollmer B. Surgical trauma: hyperinflammation versus immunosuppression? Langenbecks Arch Surg 2004; 389:475–484.
7. Rogers M, Figliomeni L, Baluchova K, et al. Do interleukin-1 polymorphisms predict the development of periodontitis or the success of dental implants? J Periodont Res 2002; 37:37–41.
8. Spyrou P, Papaioannou S, Hampson G, Brady K, Palmer R, McDonald F. Cytokine release by osteoblast-like cells cultured on implant discs of varying alloy compositions. Clin Oral Implants Res 2000; 13:623–630.
9. di Giovine FS, Poole S, Situnayake RD, Wadhwa M, Duff GW. Absence of correlation between indices of systemic inflammation and synovial fluid interleukin 1 (alpha and beta) in rheumatic diseases. Rheumatol Int 1990; 9:259–264.
10. Murray RZ, Kay JG, Sangermani DG, Stow JL. A role for the phagosome in cytokine secretion. Science 2005; 310:1492–1495.
11. Gamonal J, Acevedo A, Bascones A, Jorge O, Silva A. Levels of interleukin-1 beta, -8, and -10 and RANTES in gingival crevicular fluid and cell populations in adult periodontitis patients and the effect of periodontal treatment. J Periodontol 2000; 71:1535–1545.
12. Curtis DA, Kao R, Plesh O, Finzen F, Franz L. Crevicular fluid analysis around two failing dental implants: a clinical report. J Prosthodont 1997; 6:210–214.
13. Havemose-Poulsen A, Sorensen L, Stoltz K, Bendtzen K, Holmstrup P. Cytokine profiles in peripheral blood and whole blood cultures associated with aggressive periodontitis, juvenile idiopathic arthritis and rheumatoid arthritis. J Periodontol 2005; 76:2276–2285.
14. Dinarello CA. Proinflammatory cytokines. Chest 2000; 118:503–508.
15. Ejeil AL, Gaultier F, Igondio-Tchen S, et al. Are cytokines linked to collagen breakdown during periodontal disease progression? Periodontol 2000 2004; 35:75–100.
16. Yamazaki K, Nakajima N. Antigen specificity and T cell clonality in periodontal disease. Periodontol 2000 2004; 35:75–100.
17. Hou R, Goloubeva O, Neuberg DS, Strominger JL, Wilson SB. Interleukin-12 and interleukin-2-induced invariant natural killer T-cell cytokine secretion and perforin expression independent of T-cell receptor activation. Immunology 2003; 110:30–37.
18. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 1989; 84:1045–1049.
19. Möller A. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. Odontol Tidskrift 1966; 74(Suppl 5):1–380.
20. Slots J. Selective medium for isolation of *Actinobacillus actinomycetemcomitans*. J Clin Microbiol 1982; 15:606–609.

21. Slots J. Rapid identification of important periodontal microorganisms by cultivation. Oral Microbiol Immunol 1986; 1:48–57.
22. Parra B, Slots J. Detection of human viruses in human periodontal pockets using polymerase chain reaction. Oral Microbiol Immunol 1996; 5:289–293.
23. Contreras A, Slots J. Mammalian viruses in human periodontitis. Oral Microbiol Immunol 1996; 11:381–386.
24. Perala DG, Chapman RJ, Gelfand JA, Callahan MV, Adams DF, Lie T. Relative production of IL-1β and TNF-α by mononuclear cells after exposure to dental implants. J Periodontol 1992; 63:426–430.
25. Wang JY, Wicklund BH, Gustilo RB, Tsukayama DT. Titanium, chromium and cobalt ions modulate the release of bone-associated cytokines by human monocytes/macrophages in vitro. Biomaterials 1996; 17:2233–2240.
26. Sedarat C, Harmand MF, Naji A, Nowzari H. In vitro kinetic evaluation of titanium alloy biodegradation. J Periodontol Res 2001; 36:269–274.
27. Grayson R, Douglas CW, Heath J, Rawlinson A, Evans GS. Activation of human matrix metalloproteinase 2 by gingival crevicular fluid and *Porphyromonas gingivalis*. J Clin Periodontol 2003; 30:542–550.
28. Garlet GP, Martins W Jr, Fonseca BA, Ferreira BR, Silva JS. Matrix metalloproteinases, their physiological inhibitors and osteoclast factors are differentially regulated by the cytokine profile in human periodontal disease. J Clin Periodontol 2004; 31:671–679.
29. Chang YC, Yang SF, Lai CC, Liu JY, Hsieh YS. Regulation of matrix metalloproteinase production by cytokines, pharmacological agents and periodontal pathogens in human periodontal ligament fibroblast cultures. J Periodontal Res 2002; 37:196–203.
30. Fernandez MC, Marucha PT, Rojas IG, Walters JD. The role of protein kinase C and calclum in induction of human polymorphonuclear leukocyte IL-1 beta gene expression by GM-CSF. Cytokine 2000; 12:445–449.
31. Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljenberg B, Thomsen P. The soft tissue barrier at implants and teeth. Clin Oral Implants Res 1991; 2:81–90.
32. Stashenko P, Jandinsky JJ, Fujiyoshi P, Rynar J, Socransky SS. Tissue levels of bone resorptive cytokines in periodontal disease. J Perio 1991; 62:504–509.
33. Ishihara Y, Nishihara T, Kuroyanagi T, et al. Gingival crevicular interleukin-1 and interleukin-1 receptor antagonist levels in periodontally healthy and diseased sites. J Periodont Res 1997; 32:524–529.