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ORIGINAL ARTICLE

Microbiological and host factors are involved in promoting the periodontal failure of metaloceramic crowns

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Abstract This study was aimed at looking into the microbiological/inflammatory parameters predicting the periodontal success/failure of fixed prostheses. Microbiological and inflammatory patterns were studied at 102 sites having metaloceramic crowns in place from 3 to 6 years and divided in healthy sites (HS), gingivitis affected (MG), and periodontitis affected (PB). Total bacterial flora and selected indicator species in subgingival plaque were quantified by quantitative real-time PCR. The concentrations of IL-1β, IL-6, and TNF-α were determined in gingival crevicular fluid (GCF) by enzyme-linked immunosorbent assays. The experimental sites showed no significant difference with respect to the age and gender of the patients and to the position of the crown margins. Poor marginal adaptation was significantly higher in MG and PB. The total amounts of bacteria per probing depth showed no significant differences among the three groups and their controls, while both MG and PB sites showed altered patterns in the distribution of specific bacteria. Both MG and PB sites showed significantly higher levels of inflammatory cytokines in GCF. The control teeth of PB subjects showed significantly higher levels of IL-1β as compared to other control sites. Data confirm that the application of metaloceramic crowns is a factor of risk for the development of gingival/periodontal inflammation. This risk is possibly associated with microbiological and host factors that predispose to the onset of periodontal alterations at sites reconstructed with metaloceramic crowns. These factors, once their role is confirmed by longitudinal studies, could be used to set up rapid tests to early predict the onset of periodontal disease at reconstructed sites.

Keywords Metaloceramic crown · Periodontitis · Bacteria · Inflammation · Factors of risk

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Introduction

A dental prosthesis is successful only if the restoration remains in place over a long time and without causing any disease [1–5]. However, many evidences that show the existence of a close relationship between the presence of fixed prosthetic restorations and an enhanced risk of periodontal infections have been collected [2, 6]. A lot of researches were consequently addressed at studying different dental materials with respect to their susceptibility to colonization by selected bacterial species [7–9], their surface characteristics, and many technical aspects related to the possibility to reconstruct normal dental anatomy [1, 5]. These studies, nevertheless, frequently do not adequately classify periodontal conditions and confuse mere gingival inflammation and periodontal breakdown, ultimately possibly leading to ambiguous conclusions [6, 10–12]. Moreover, they do not elucidate if any factor exists that predisposes patients to the onset of periodontitis on dental elements that are restored with a fixed prosthesis. The disclosure of such

factors could prove to be of high value in identifying patients that are more prone to undergo periodontal infections as a consequence of fixed prostheses positioning. Consequently, the availability of a limited number of analytical parameters that can be easily, rapidly, and inexpensively determined directly by the dentist to reliably evaluate the presence or predict the onset of periodontal or other complications at a prosthetic site would be of utmost importance in clinical dentistry. Since the onset of both marginal gingivitis and periodontal breakdown are a consequence of the activity of dental plaque and are associated with tissue damage and inflammation, it is reasonable to look for microbiological and/or inflammatory parameters as indicators or predictors of these complications to evaluate the possibility to develop innovative rapid diagnostic procedures that are able to help the dentists in their distinction.

This study was aimed to evaluate the correlations between microbiological/inflammatory parameters and the clinical indicators of the periodontal success/failure of fixed prostheses that had been in place from 3 to 6 years before the study in order to look for parameters that can be considered as predictors of failure.

Materials and methods

Patients and controls

To be included in the study, the subjects should: (a) have between one to three teeth with metaloceramic crowns in place from 3 to 6 years before the study, (b) have the contralateral teeth without significant restorations (i.e., without restorations involving any area in direct contact with the gingival margin), (c) have no clinical and radiographic evidence of chronic periodontal disease affecting the teeth other than the ones with metaloceramic crowns, (d) have not received any antibiotic therapy in the previous 60 days, (e) have no systemic disorder predisposing to infections, and (f) not be pregnant or breast-feeding.

Seventy-four individuals, 35 male (47.3%) and 39 female (52.7%), of age 29 to 47 years gave their written informed consent and were enrolled in the study.

From these 74 individuals, 102 pairs of teeth were studied (1.38 teeth/patient), namely, 10 pairs of incisors (9.8%), 7 pairs of canines (6.9%), 49 pairs of premolars (48.0%), and 36 pairs of molars (35.3%).

Clinical evaluations The periodontal and dental conditions of all the patients and their compliance with the inclusion criteria were evaluated by a single skilled examiner. The absence of periodontal disease affecting the teeth other than the ones with metaloceramic crowns was evaluated as: (a) the absence of probing depths ≥4 mm on six sites (distobuccal, midbuccal, mesiobuccal, distolingual, midlingual, and mesiolingual) for each tooth, (b) the absence of radiographic evidence of either vertical or horizontal bone loss, (c) the radiographic evidence of the presence of the crestal lamina dura, (d) the localization of the alveolar bone level within 1 to 2 mm of the cementum enamel junction area, and (e) the absence of clinical evidences of inflammation and of bleeding on probing. The periodontal conditions of the experimental and control teeth as well as the clinical conditions of the crowns were evaluated separately by two distinct and skilled examiners who were blind to the parameters. The periodontal conditions were clinically assessed in terms of visible plaque index (VPI), gingival bleeding index (GBI), and probing pocket depth (PPD). Each tooth, either experimental or control, was evaluated separately at six sites for each parameter. PPD was evaluated by a Williams periodontal probe. Radiographic evaluations were performed assuming the cementum enamel junction area to be aligned with that of the adjacent teeth.

The clinical conditions of the crown were assessed concerning the clinical location of the crown margin (whether supragingival or subgingival) and its adaptation (whether well fit or maladapted), according to previously published criteria [11], at six sites for each tooth. Concerning the positioning of crown margins, crowns showing very different conditions at the six sites were not included in the study. Concerning marginal adaptation, any crown showing even a single maladapted site was considered as maladapted.

Following the clinical and radiographic evaluation of the experimental and control teeth, the 102 selected elements were divided in three groups: (1) 34 healthy sites (HS), showing no evidence of marginal gingivitis or periodontal breakdown, as assessed by PPD <4 mm, radiographic evidence of the presence of the crestal lamina dura, and alveolar bone level within 1 to 2 mm of the cementum enamel junction area, gingival index (GI) ≤1; (2) 31 gingivitis affected sites (MG), showing evidences of marginal gingivitis affecting the teeth with crowns, as assessed by radiographic evidence of the presence of the crestal lamina dura, and alveolar bone level within 1 to 2 mm of the cementum enamel junction area, GI >1; and (3) 37 sites with periodontal breakdown (PB), showing clinical and radiographic evidences of localized periodontal breakdown affecting teeth with crowns, as assessed by PPD ≥4 mm, radiographic evidence of the absence of the crestal lamina dura, and alveolar bone levels exceeding 2 mm of the cementum enamel junction area, GI >1.

Microbiological analyses

Microbiological samples were obtained from each site after the accurate removal of supragingival plaque. Bacteria were

collected by a sterile curette and transferred to a 2.0-ml microcentrifuge tube containing 0.5 ml of sterile DNAse-free molecular-grade water (Sigma Chemical Company). The samples were transported to the laboratory in dry ice and then stored at −80°C until processing.

Bacterial DNA was extracted from microbiological samples and from pure cultures of reference strains of the different bacterial species (*Aggregatibacter actinomycetemcomitans* DSM 8324, *Campylobacter rectus* DSM 3260, *Eubacterium saphenum* CCUG 52676, *Mogibacterium timidum* DSM 3998, *Porphyromonas gingivalis* DSM 20709, *Prevotella intermedia* DSM 20706, *Prevotella tannerae* CCUG 34292, *Slackia exigua* DSM15923, *Tannerella forsythia* CCUG 21028, *Staphylococcus aureus* ATCC 6538, *Streptococcus gordonii* DSM 6777, *Streptococcus oralis* DSM 20627, *Streptococcus sanguinis* DSM 20567, *Streptococcus salivarius* DSM 20560) using the NucleoSpin genomic DNA purification kit (Macherey-Nagel GmbH, Düren, Germany). Samples were centrifuged at 12,000×*g* at 4°C, and the bacterial pellet was processed for bacterial DNA extraction. In order to enable complete cell lysis, bacteria were incubated in 20 mM Tris–HCl, 2 mM EDTA, 1% Triton X-100, pH 8 supplemented with 20 mg/ml lysozyme, and 0.2 mg/ml lysostaphin for 60 min at 37°C. Proteinase K was then added, and samples were incubated at 56°C until complete lysis was obtained. Following lysis, total DNA was purified according to the instructions of the manufacturer.

The quantification of the total amount of bacteria and of the specific amounts of selected bacterial species in samples was performed by real-time PCR and was normalized for each site to probing depth and expressed as log femtogram DNA per microliter per millimeter of probing depth. The total amounts of bacteria were evaluated using the 16S rRNA gene universal primers 357F and 907R [13, 14] using the Maxima® SYBR Green/Fluorescein qPCR Master Mix (Fermentas Life Sciences) according to the instructions of the manufacturer. Cycling conditions were performed as described previously [14] and were undertaken using an iQ5 real-time PCR system (Bio-Rad Laboratories). Amplification data were analyzed using iCycler iQ software (Bio-Rad Laboratories). A quantitative analysis was performed following the construction of a standard curve using the universal primers against a serial dilution of a quantified mix of purified bacterial genomes constructed within the laboratory.

The quantification of specific bacteria in the samples was performed as described previously [15] using the indicated primers with the addition of primers 16Saur QF (ctgtgcacatcttgacggtacc) and 16Saur QR (cgcttgccacctacg tattacc) for the detection of *S. aureus*. A quantitative analysis was performed following the construction of species-specific standard curves using each couple of primers against a serial dilution of the corresponding genomic DNA.

Evaluation of inflammatory mediators

The concentrations of IL-1β, IL-6, and TNF-α in the gingival crevicular fluid (GCF) of patients were determined by high-sensitivity enzyme-linked immunosorbent assays (Quantikine HS, R&D System). GCF was collected from the buccal aspect of each experimental and control tooth. The area adjacent to the tooth to be sampled was isolated by means of cotton rolls, supragingival plaque was carefully removed with a curette, and the tooth was gently dried with air. Two Periopaper Strips (Interstate Drug Exchange, Amityville, NY, USA) were inserted at the entrance of the gingival crevice and kept there for 30 s, as described earlier [16].

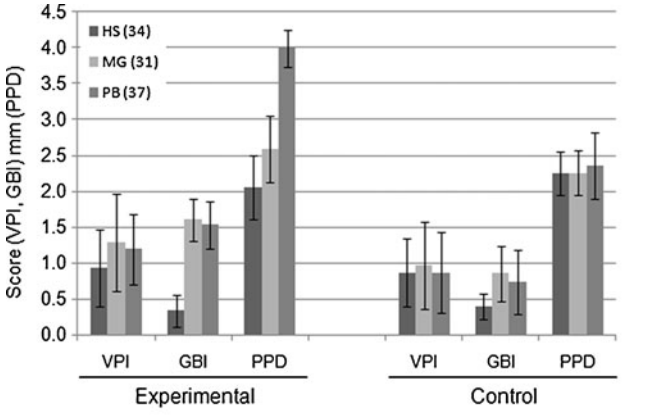
The strips from each tooth were then combined and placed in 1 ml of PBS containing a protease inhibitor cocktail (IBI Scientific, Peosta, IA, USA) transported to the laboratory in dry ice and then stored at −80°C until processing. Before being processed for cytokine analysis, each vial was vortexed for 3 min, and samples were clarified by centrifugation at 20,000×*g* for 5 min at 4°C. Analyses to determine the concentration of each cytokine were performed twice in triplicate, according to the instructions of the manufacturer. Results were calculated using the standard curves created in each assay. The concentrations of the cytokine were expressed as picogram per milliliter, assuming data as indicating the total amount of each cytokine per tested site without normalizing to GCF volume variations.

Statistical analysis

The evaluation of significance of the differences in clinical, microbiological, and inflammatory parameters among the groups of experimental and control sites was performed by Student's *t* tests and by Fisher exact test, performed at a significance level of $P \leq 0.01$, using statistical analysis tools of the Microsoft Excel Software and the online resource available at http://www.people.ku.edu/~preacher/.

Results

Clinical conditions Overall, 102 teeth restored with metal-ceramic crowns and the corresponding non-restored control teeth from 74 subjects were studied. No significant difference existed in the three groups with respect to age and gender of the patients. Patients of the MG and PB groups had a higher number of metaloceramic crowns (i.e., 1.55 and 1.68 crowns/patient, respectively) as compared to



Values of *P* according to Student T test						
VPI	GBI	PPD		VPI	GBI	PPD
0.02	$3.1x10^{-28}$	$1.1x10^{-5}$	HSvMG	0.46	$2.8x10^{-8}$	0.87
0.03	$6.4x10^{-27}$	$8.9x10^{-34}$	HSvPB	0.99	$1.3x10^{-4}$	0.22
0.48	0.37	$5.4x10^{-24}$	MGvPB	0.49	0.22	0.30

Fig. 1 Results of the evaluation of visible plaque index (*VPI*), gingival bleeding index (*GBI*), and periodontal probing depth (*PPD*) of 102 couples of teeth (*experimental*=teeth with metaloceramic crowns; *control*=contralateral control teeth) divided in healthy sites (*HS*), marginal gingivitis sites (*MG*), and periodontal breakdown sites (*PB*) according to clinical the evaluation of experimental sites. Data are reported as means (±standard deviation) for each group. Evaluations were performed at six sites for each tooth. Individual values of *P*, obtained by Student's *t* test performed by comparing the values of each group versus the other two, are reported

HS (i.e., 1.06 crowns/patient), further supporting the potentially negative effect of the presence of crowns for periodontal conditions. Seventy-seven of these crowns (74.0%) had subgingival margins and no significant difference existed in marginal positioning among the three groups. The marginal adaptation of crowns was judged good in 86 cases (84.3%). Poor marginal adaptation of crowns was higher in both MG and PB (22.6% and 16.2%, respectively) as compared to HS (8.8%), although the differences were not statistically significant (P=0.09 and 0.08, respectively).

The comparability of the three groups of patients as to the accuracy of oral hygiene and the overall periodontal status is shown by the absence of any difference in the mean VPI and PPD values among the groups of control sites. The analysis of clinical parameters showed that both MG and PB experimental sites showed higher mean VPI values as compared to HS. Although differences were not highly significant, no differences among the groups were evident at the control sites (Fig. 1). Both MG and PB sites showed higher mean GBI values as compared to HS; differences were evident and significant at both experimental and control sites (Fig. 1).

Microbiological analyses The microbiological samples of subgingival plaque obtained from both experimental and control teeth, after the removal of supragingival plaque, were analyzed by real-time PCR for the quantification of the total amount of bacteria and of the specific amounts of selected bacterial species. Quantitative data were normalized for each site to probing depth and expressed as log femtogram DNA per microliter per probing depth millimeter. The total amounts of bacteria per probing depth obtained from experimental and control teeth classified in three categories (i.e., HS, MG, and PB) were in the range 3.45±0.09–4.12±0.32 log fg DNA/μL per probing depth mm (Fig. 2). No significant differences were observed among the groups and between experimental and control teeth. No sample resulted positive for *A. actinomycetemcomitans*.

As expected, several bacterial species were not detected in samples from HS and MG, including *E. saphenum*, *M. timidum*, *P. gingivalis*, *P. tannerae*, and *T. forsythia* (Table 1). No statistically significant difference existed in the percentages of PCR positives for any of the searched bacterial species between experimental and control sites in HS and MG (Table 1). The percentages of PCR positives for *C. rectus*, *E. saphenum*, *M. timidum*, *P. gingivalis*, *P. intermedia*, *P. tannerae*, *S. exigua*, and *T. forsythia* were significantly higher in experimental PB sites as compared to control sites (Table 1). The distribution of isolates of the different species in the three groups between sites with crowns showing maladapted margins and sites with crowns showing well-fitted margins, as compared to the reference ratio of the two classes, is reported in Table 2. Results show that although no statistically significant difference is evident due to the exiguity of the sample, *S. aureus* isolates were more frequent in PB sites, showing well-fitted margins as compared to the corresponding sites with maladapted margins.

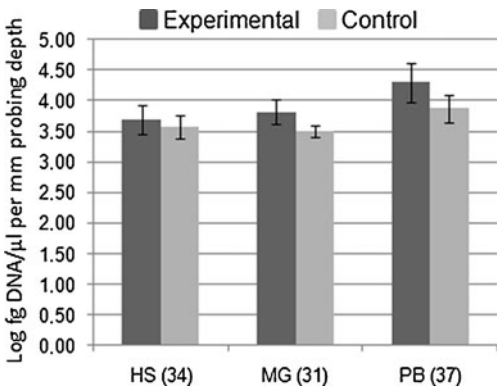


Fig. 2 Amounts of total bacterial DNA extracted from microbiological samples of 102 couples of teeth (*experimental*=teeth with metaloceramic crowns; *control*=contralateral control teeth) divided in healthy sites (*HS*), marginal gingivitis sites (*MG*), and periodontal breakdown sites (*PB*) according to clinical the evaluation of experimental sites. Data are reported as means (±standard deviation) of log fg DNA/μl per mm of probing depth for each group

Table 1 Percentages of experimental and control sites positive for the detection of species specific DNA sequences of selected bacterial species

	Percentage of sites positive for species specific DNA (Nr)								
	HS (34)			MG (31)			PB (37)		
Species	Exp	Ctrl	P^a	Exp	Ctrl	P^a	Exp	Ctrl	P^a
C. rectus	35.3 (12)	23.5 (8)	0.43	35.5 (11)	25.8 (8)	0.58	91.9 (34)	29.7 (11)	*<0.01**
E. saphenum	0.0 (0)	0.0 (0)	ND	0.0 (0)	0.0 (0)	ND	89.2 (33)	0.0 (0)	*<0.01**
M. timidum	0.0 (0)	0.0 (0)	ND	0.0 (0)	0.0 (0)	ND	86.5 (32)	0.0 (0)	*<0.01**
P. gingivalis	0.0 (0)	0.0 (0)	ND	0.0 (0)	0.0 (0)	ND	67.6 (25)	0.0 (0)	*<0.01**
P. intermedia	23.5 (8)	14.7 (5)	0.54	41.9 (13)	25.8 (8)	0.28	100.0 (37)	29.7 (11)	*<0.01**
P. tannerae	0.0 (0)	0.0 (0)	ND	0.0 (0)	0.0 (0)	ND	21.6 (8)	0.0 (0)	*<0.01**
S. exigua	17.6 (6)	17.6 (6)	1	22.6 (7)	22.6 (7)	1	70.3 (26)	21.6 (8)	*<0.01**
T. forsythia	0.0 (0)	0.0 (0)	ND	0.0 (0)	0.0 (0)	ND	56.8 (21)	0.0 (0)	*<0.01**
S. aureus	14.7 (5)	14.7 (5)	1	29.0 (9)	29.0 (9)	1	24.3 (9)	24.3 (9)	1
S. gordonii	100.0 (34)	100.0 (34)	1	100.0 (31)	100.0 (31)	1	100.0 (37)	100.0 (37)	1
S. oralis	100.0 (34)	100.0 (34)	1	100.0 (31)	100.0 (31)	1	100.0 (37)	100.0 (37)	1
S. sanguinis	100.0 (34)	100.0 (34)	1	100.0 (31)	100.0 (31)	1	100.0 (37)	100.0 (37)	1
S. salivarius	100.0 (34)	100.0 (34)	1	100.0 (31)	100.0 (31)	1	100.0 (37)	100.0 (37)	1

Italicized characters indicate statistically significant differences

*$P<0.01$

[a] A two-tailed Fisher exact test was applied

The analysis of quantitative microbiological data showed the existence of several statistically significant differences between experimental and control MG and PB sites (Table 3). In fact, in MG, *S. gordonii*, *S. salivarius*, and *S. aureus* were detected in significantly higher amounts from experimental than from control sites, while *S. sanguinis* and *S. oralis* were significantly more abundant in control sites (Table 3). All of the studied bacteria were detected in significantly different amounts between experimental and control sites in PB (Table 3).

The comparison of the amounts of specific bacteria detected from the experimental sites of different clinical conditions showed that while *S. gordonii* and *S. salivarius* were significantly more abundant in MG than in HS, *S. sanguinis* and *S. oralis* were significantly more abundant in HS and no significant difference was observed for *S.*

Table 2 Numbers and ratios of isolates of selected bacterial species from experimental sites with crowns showing maladapted margins/crowns showing well-fitted margins compared with reference ratios calculated as the number of sites with crowns showing maladapted margins/crowns showing well-fitted margins in each group

	Number (ratio) of isolates from maladapted/well-fit crowns		
	HS (34)	MG (31)	PB (37)
Reference ratio	3/31 (0.10)	7/24 (0.29)	6/31 (0.19)
Species			
C. rectus	1/11 (0.09)	3/8 (0.37)	6/28 (0.21)
E. saphenum	NI	NI	6/27 (0.22)
M. timidum	NI	NI	5/24 (0.21)
P. gingivalis	NI	NI	5/20 (0.25)
P. intermedia	1/7 (0.14)	3/10 (0.30)	6/31 (0.19)
P. tannerae	NI	NI	2/6 (0.33)
S. exigua	1/5 (0.20)	2/5 (0.40)	5/21 (0.24)
T. forsythia	NI	NI	4/17 (0.24)
S. aureus	1/4 (0.25)	2/7 (0.29)	1/8 (0.12)

HS healthy sites, *MG* marginal gingivitis sites, *PB* periodontal breakdown sites, *NI* no isolation from the corresponding group

Table 3 Proportions of *C. rectus*, *E. saphenum*, *M. timidum*, *P. gingivalis*, *P. intermedia*, *P. tannerae*, *S. exigua*, *T. forsythia*, *S. aureus*, *S. gordonii*, *S. oralis*, *S. sanguinis*, and *S. salivarius* in subgingival plaque obtained from subgingival sites of teeth with EXP or without CTRL metaloceramic crowns and characterized by different periodontal conditions

Species	Percentual proportion of the selected bacterial species in subgingival plaque (±SD)						Value of *P* for comparison in EXP		
	HS (34)		MG (31)		PB (37)		HS vs MG	HS vs PB	MG vs PB
	EXP	CTRL	EXP	CTRL	EXP	CTRL			
C. rectus	0.54 (0.09)	0.48 (0.08)	0.60 (0.15)	0.52 (0.10)	1.11 (0.21)	0.47 (0.06)[a]	0.39	<0.01	<0.01
E. saphenum	ND	ND	ND	ND	0.48 (0.13)	ND[a]		<0.01	<0.01
M. timidum	ND	ND	ND	ND	0.10 (0.03)	ND[a]		<0.01	<0.01
P. gingivalis	ND	ND	ND	ND	0.70 (0.14)	ND[a]		<0.01	<0.01
P. intermedia	0.09 (0.03)	0.11 (0.04)	0.09 (0.03)	0.10 (0.02)	0.31 (0.07)	0.16 (0.03)[a]	0.48	<0.01	<0.01
P. tannerae	ND	ND	ND	ND	0.23 (0.15)	ND[a]		0.02	0.02
S. exigua	1.12 (0.31)	0.98 (0.17)	1.88 (0.56)	1.12 (0.18)	1.74 (0.34)	1.14 (0.19)[a]	0.03	0.01	0.49
T. forsythia	ND	ND	ND	ND	2.11 (0.40)	ND[a]		<0.01	<0.01
S. aureus	0.07 (0.02)	0.06 (0.08)	0.17 (0.04)	0.05 (0.02)[a]	0.19 (0.06)	0.08 (0.02)[a]	<0.01	<0.01	0.22
S. gordonii	0.05 (0.03)	0.06 (0.05)	0.22 (0.03)	0.09 (0.02)[a]	0.23 (0.06)	0.10 (0.04)[a]	<0.01	<0.01	0.18
S. oralis	7.09 (0.74)	7.28 (0.59)	2.69 (0.72)	6.83 (0.67)[a]	0.54 (0.12)	7.07 (0.56)[a]	<0.01	<0.01	<0.01
S. sanguinis	0.45 (0.06)	0.55 (0.09)	0.18 (0.04)	0.51 (0.05)[a]	0.18 (0.03)	0.57 (0.08)[a]	<0.01	<0.01	0.31
S. salivarius	0.36 (0.16)	0.34 (0.11)	0.64 (0.08)	0.30 (0.03)[a]	0.68 (0.12)	0.31 (0.03)[a]	<0.01	<0.01	0.43

HS healthy site, *MG* marginal gingivitis site, *PB* periodontal breakdown site, *EXP* experimental site with metaloceramic crown, *CTRL* contralateral control site without any artificial crown, *ND* not detected

[a] Indicates significant differences between experimental and control sites, with a Student's *t* test yielding values of $P \leq 0.01$

aureus, *P. intermedia*, and *C. rectus* (Table 3). The amounts of *S. exigua* detected in HS and MG were different although the differences were not highly significant (Table 3). All of the studied bacteria except *S. aureus* were recovered in significantly different amounts from PB and HS experimental sites (Table 3); the differences were also not highly significant for *P. tannerae*. Significant differences were also detected in the amounts of specific bacteria detected between MG and PB sites (Table 3).

Evaluation of inflammatory mediators High-sensitivity enzyme-linked immunosorbent assays used to evaluate the concentration of IL-1β, IL-6, and TNF-α in the GCF obtained from the studied teeth confirmed that the presence of either MG and PB is associated with a significant enhancement of the secretion of inflammatory cytokines with GCF as compared to HS and to contralateral controls (Fig. 3). Although clinical indices suggest the absence of any significant periodontal pathology affecting the con-

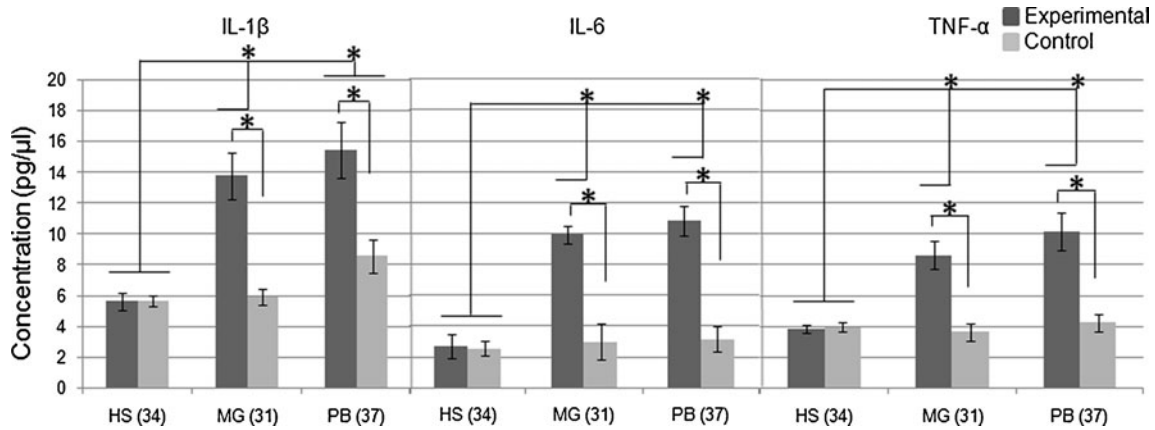


Fig. 3 Concentrations of IL-1β, IL-6, and TNF-α detected by immunoenzymatic methods in the gingival crevicular fluid obtained from 102 couples of teeth (*experimental*=teeth with metaloceramic crowns; *control*=contralateral control teeth) divided in healthy sites (*HS*), marginal gingivitis sites (*MG*), and periodontal breakdown sites (*PB*) according to the clinical evaluation of experimental sites. Data are reported as means (±standard deviation) for each group. An *asterisk* indicates significant differences between groups as demonstrated by Student's *t* test performed at a significance level of 0.01

trol teeth of MG and PB, significant differences were observed in the concentration of IL-1β detected in PB as compared to both HS ($P=4.8\times10^{-3}$) and to a minor extent MG ($P=0.02$) (Fig. 3).

Discussion

In dentistry, prosthetic restorations are generally intended for long-term durability without causing any damage to the tooth and surrounding tissues [1–5]. Although an obvious assumption, many are the obstacles that are encountered in the way towards a complete integration of the artificial crown in the dental/periodontal environment. In recent years, many evidences demonstrating that the presence of a fixed prosthetic restoration enhances the risk of the onset of periodontal lesions have been collected [2, 6]. The awareness of the importance of an abnormal subgingival microbiota in the etiology of periodontal disease and that of adhesion to the hard tissues in dental plaque formation inspired studies aimed to identify materials that were less prone to bacterial colonization [7–9] and more suitable to reconstruct normal dental anatomy [1, 5]. Although useful to develop optimized materials and techniques, these studies often failed to take into consideration important biological and clinical aspects of the problem and consequently failed to completely unveil the question [6, 10–12].

While no doubt exists that a fixed prosthetic restoration should be constructed with the most suitable materials available, also to minimize the biological effects due to material interactions with oral tissues [17, 18], paying attention to the position and adaptation of margins, it is a common clinical observation that some patients are more prone to develop periodontal infections at sites where artificial crowns are positioned even if these crowns show no apparent defect. The reasons of this are unclear although the existence of subjective predisposing conditions is clearly suspected. Data presented in this paper confirm that, in a group of periodontally healthy subjects characterized by comparable conditions of oral hygiene at natural teeth and by a comparable general periodontal status, the application of metaloceramic crowns is a factor of risk for the development of inflammation, limited to the gingiva or diffused to the periodontium and causing localized damage to periodontal tissues. The risk is generally believed to be mainly dependent on the interference of the crown with normal hygiene that causes excessive accumulation of plaque, promotes colonization by pathogenic bacteria, and favors inflammation. Our data confirm only in part to such a scenario. In fact, in the studied population, including periodontally healthy subjects who had one to three teeth with metaloceramic crowns in place from 3 to 6 years and the contralateral teeth without significant restorations, while control teeth showed an overall comparable periodontal and microbiological situation, experimental teeth showed different clinical situations ranging from periodontal health to localized periodontitis. Based on clinical conditions, experimental teeth were divided into three groups and characterized from the microbiological and inflammatory points of view. As expected, the presence of localized periodontal pathology positively correlated with the number of metaloceramic crowns per subject and with the presence of poor marginal adaptation of crowns, but not with the position of crown margins. Nevertheless, only 22.6% and 16.8% of MG and PB sites were characterized by poor margin adaptation, suggesting that other non-disclosed factors may influence the periodontal conditions at these sites.

Both experimental and control sites were analyzed at the microbiological level to search for microbiological indicators of these failures. We decided to evaluate the subgingival microbial flora alone since this is believed worldwide to be related to periodontal status. Subgingival plaque was analyzed by quantitative real-time PCR assays, enabling to quantify the total subgingival microbial flora, irrespective of its cultivability, and in parallel the presence of selected microorganisms associated with different periodontal conditions. Although the overall amounts of subgingival plaque that were recovered from both MG and PB sites were higher than those from HS, this was only a consequence of a larger sampling area induced by enhanced PPD. In fact, when quantitative data on bulk bacterial DNA recovered from subgingival samples were normalized to PPD, comparable values were obtained from all of the groups. These observations are in accordance with data of literature, demonstrating that when subgingival plaque is quantitatively normalized to PPD no significant difference exists between healthy and periodontally affected sites [15]. These data suggest that the presence of artificial crowns does not induce an excessive accumulation of subgingival plaque and that the consequent accumulation of subgingival plaque does not play a role in the onset of gingival and periodontal pathologies at these sites.

Quantitative data on the presence of selected bacterial species in subgingival samples from experimental and control teeth showed that all control sites and experimental HS sites were characterized by the presence of bacterial species typically associated with healthy periodontium [15], while both MG and PB sites were characterized by the presence of a qualitatively altered subgingival microbiota. In fact, while MG sites were characterized by an altered balance of streptococcal species, in the absence of typical periodontopathogens, PB sites were characterized by the presence of many species associated with periodontal disease [15] and by a reduced presence of streptococci.

Although no specific microbiological character was associated with MG or PB sites characterized by good marginal adaptation, this is possibly due to the limited number of samples that were analyzed. In fact, the analysis of ratios obtained by dividing the number of isolates from sites with maladapted margins for the number of isolates from sites with well-fitted margins revealed that in PB only *S. aureus* showed a ratio that was much lower than the reference ratio obtained by dividing the total number of sites of each class (maladapted and well fitted) in the group, while *P. tannerae* appeared to be more frequently associated with periodontal lesions of elements with maladapted margins. Microbiological analyses, in consequence, while confirming the clinical classification of sites, were only limitedly helpful in disclosing any factor predicting the onset of localized gingival/periodontal lesions. The set of bacterial species detected in samples included also *S. aureus* although it is not a typical component of the periodontal flora. We searched *S. aureus* within a program dedicated to the study of the role of the oral cavity as a possible reservoir for *S. aureus* carriage. While these aspects are not in the scopes of this work, it must be noted that the amounts of *S. aureus* detected in both MG and PB differed significantly from those detected from HS and from the corresponding control sites. The isolation of *S. aureus* from periodontally diseased sites is not new, and the role of this microorganism in the pathogenesis of lesions is debated in the literature [19–21]. In fact, this microorganism is endowed with a wide armamentarium of adhesins enabling the colonization of hard surfaces and toxins and secreted factors that can actively and efficiently manipulate inflammation and immune responses of the host. These abilities could prove relevant to colonize the ceramic surfaces, expand to subgingival sites, and cooperate in triggering an altered reactivity of the host in turn promoting modifications of the subgingival flora opening the way to disease.

The data of the recent literature suggest that many bacterial species have evolved systems that enable them to sense the inflammatory environment of the host and react consequently by modulating the expression of selected sets of genes in accordance [22]. This ability is believed to have a relevant role in the process of adaptation to pathology and is also believed to be able to promote genetic evolution of microorganisms towards higher pathogenicity [23]. The presence of an altered inflammatory milieu is also known to promote bacterial biofilm growth of some bacterial species [24]; thus, the higher VPI scores obtained at both experimental and control sites of MG and PB could be a consequence of the presence of higher levels of IL-1β and other cytokines rather than its cause.

The existence of factors of the host that predispose to the onset of periodontal alterations at sites reconstructed with metaloceramic crowns is suggested by data on the concentration of inflammatory mediators in the GCF of the studied sites. In fact, while the existence of significant differences in the levels of the studied mediators between HS and both MG and PB is obvious [25], the existence of a significant difference in the level of IL-1β between the control sites of HS and PB was not expected and, in the absence of clinical evidences of inflammation at these sites, suggests the existence of an altered reactivity of unknown origin. Thus, it can be hypothesized that intrinsic (genetic?) or extrinsic (microbiological?) factors could be responsible to induce an altered reactivity of the host that predisposes subjects to the onset of periodontal lesions at sites reconstructed with metaloceramic crowns. To clarify these aspects, longitudinal studies that monitor a significant number of sites from the microbiological and inflammatory point of view are needed. It is moreover difficult to explain why IL-1β, but not the other two studied cytokines, showed enhanced levels at these sites. Further work will be necessary to elucidate this aspect by investigating on more detailed profiles of inflammatory mediators in comparable clinical conditions. Although cytotoxicity could play the role of a third factor inducing an altered reactivity of tissues [17, 18, 26], the data of this study cannot contribute to investigate this aspect since no information were available on materials used in building the studied crowns. Data obtained in this work could prove to be a useful starting point in the search for molecular markers able to predict and/or early detect pathologic conditions favoring the onset of periodontal disease at sites reconstructed by fixed prostheses.

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Conflicts of interest The authors declare that they have no conflicts of interest.

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