Effect of Intracrevicular Restoration Margins on Peri-implant Health: Clinical, Biochemical, and Microbiologic Findings Around Esthetic Implants up to 9 Years

Catherine Giannopoulou, Dr Med Dent[1]/Jean-Pierre Bernard, PD Dr Med[2]/
Daniel Buser, Prof Dr Med Dent[3]/Anne Carrel[4]/Urs C. Belser, Prof Dr Med Dent[5]

Purpose: *The aim of the present study was to evaluate longitudinally the stability of a cohort of esthetic implants that had been in function for at least 1 year prior to the baseline examination.* ***Materials and Methods:*** *Sixty-one maxillary anterior ITI implants in 45 systemically healthy patients, supporting single crown restorations, were randomly selected and examined. Clinical, microbiologic, and biochemical parameters were recorded at baseline and again after 3 years. Clinical examination included Plaque Index, Gingival Index, bleeding on probing, probing pocket depth (PPD), distance between implant shoulder and mucosal margin (DIM), and mobility. Dark-field microscopy and immunofluorescence were used to evaluate the bacteria morphotypes and the presence of 5 specific pathogenic bacteria, respectively. Peri-implant crevicular fluid (PICF) was collected at the mesial and distal sites of each implant, and total amounts of 3 biochemical markers were assessed: alkaline phosphatase was measured by using p-nitrophenyl-phosphate as substrate, elastase activity was measured by the use of a low-molecular-weight fluorogenic substrate, and the inhibitor α2-macroglobulin (α2M) was measured by enzyme-linked immunosorbent assay.* ***Results:*** *The only statistically significant differences between baseline and follow-up examination concerned PPD and DIM measurements, which increased slightly. The remainder of the clinical measurements and almost all of the microbiologic and biochemical parameters did not change significantly. Furthermore, no associations were observed between the above results and the number of years that implants had been in function.* ***Discussion and Conclusions:*** *Based on an observation period of 4 to 9 years (mean 6.8 years at the time of the follow-up examination), it can be concluded that in patients with appropriate oral hygiene, the intracrevicular position of the restoration margin does not appear to adversely affect peri-implant health and stability.* (Int J Oral Maxillofac Implants 2003;18:173–181)

Key words: *bacteria, crown, dental implants, fixed partial denture, implant-supported dental prosthesis, intracrevicular margins, peri-implant crevicular fluid*

[1]Assistant Professor, Department of Periodontology and Oral Pathophysiology, School of Dental Medicine, University of Geneva, Switzerland.
[2]Associate Professor, Department of Stomatology and Oral Surgery, School of Dental Medicine, University of Geneva, Switzerland.
[3]Professor, Department of Oral Surgery and Stomatology, University of Bern, School of Dental Medicine, Bern, Switzerland.
[4]Laboratory Technician, Department of Preventive Dentistry, School of Dental Medicine, University of Geneva, Switzerland.
[5]Professor, Department of Prosthetic Dentistry, School of Dental Medicine, University of Geneva, Switzerland.

Reprint requests: Dr Catherine Giannopoulou, Department of Periodontology and Oral Pathophysiology, University of Geneva, School of Dental Medicine, 19 rue Barthélemy-Menn, 1205 Geneva, Switzerland. Fax: +41-22-7811297. E-mail: Ekaterini.Giannopoulou@medecine.unige.ch

In recent decades, the clinical replacement of natural teeth by osseointegrated implants has represented one of the most significant advances in restorative dentistry. Since then, numerous studies of various clinical indications have documented high survival and success rates with respect to specific criteria.[1–7] Along with osseointegration and restoration of function, the patient's subjective satisfaction is a key element of the success of implant therapy. Especially when the implant is located in the anterior part of the oral cavity, an essential part of the therapy aims to create appropriate conditions, so that finally the implant-supported prosthesis cannot be distinguished from the adjacent natural teeth. This is typically accomplished by locating the crown margin

submucosally. Furthermore, several procedures have been developed, including novel bone augmentation, connective tissue grafting, and reconstruction of lost papillary tissue. Recently, an ITI Consensus Conference deemed it appropriate to locate the implant shoulder submucosally so as to respond to natural esthetic demands.[8] As the current implant design, in contrast to the scalloped cementoenamel junction (CEJ), features a straight, horizontal, "rotation-symmetric" restorative interface, interproximal crown margins are often located several millimeters submucosally and are thus difficult to reach during the patient's routine oral hygiene efforts.[9,10]

Currently, it is generally accepted that the final implant shoulder position for esthetic fixed single crown restorations can be determined by the location of the CEJ of the adjacent teeth and by the level of the free gingival margin at the vestibular aspect of these same teeth. This means that the shoulder is positioned 1 to 2 mm more apically to the labial CEJ of the adjacent teeth[9] when using ITI implants. However, the noticeable esthetic progress made in this type of implant restoration has been the result of recent developments in the absence of extensive long-term documentation. Because the exclusive use of clinical signs for establishing peri-implant health or disease may not be sufficient, the evaluation of additional objective parameters is needed.

A number of diagnostic tests have been utilized by clinicians to supplement clinical signs with objective methods. These tests include microbiologic monitoring, proteolytic bacterial enzyme markers, markers of tissue destruction, and finally, markers of tissue repair and regeneration. In this context, recently, peri-implant crevicular fluid (PICF) analysis has become the focus of intense investigation. Apse and coworkers[11] observed that the volume of crevicular fluid did not differ between implant sites and natural teeth, and the features of inflammation seemed to be the same around teeth and implants. In addition, the histologic arrangement of peri-implant soft tissues resembles basically that observed around natural teeth, although it also features some aspects of scar tissue.[12–15]

Last and associates[16,17] evaluated the glycosaminoglycan (GAG) content in PICF. Two GAG bands, hyaluronic acid and chondroitin 4-sulfate, were detected. In addition, PICF volume and GAG levels were higher at peri-implantitis sites when compared to healthy control sites. Eley and colleagues[18] evaluated protease activity in PICF and reported that the total activity of elastase, cathepsin, dipeptidyl peptidase, and trypsin was correlated positively with Gingival Index values and bone resorption. Finally, analysis of interleukin-1β levels (IL-1β) in diseased and healthy peri-implant tissues indicated that IL-1β might provide a means of monitoring the health status of tissues around dental implants.[19,20]

Two enzymes, elastase and alkaline phosphatase, and the inhibitor α2-macroglobulin (α2M) were shown to be associated with tissue destruction in periodontitis.[21] Elastase is a serine protease capable of degrading several functionally and structurally important proteins in the periodontium.[22] Increased gingival crevicular fluid (GCF) elastase activity has been found in periodontitis sites,[23,24] and it has been suggested that elastase activity could be a predictor of disease progression.[25,26] However, GCF elastase activity may be reduced by endogenous inhibitors such as α2M, reported to be present in both gingival tissue[27] and gingival fluid.[28]

Finally, alkaline phosphatase, an enzyme involved in bone metabolism, has been shown to be significantly elevated in active as compared to inactive sites[29] and has been suggested to be a predictor of current or future disease activity.[30] Recently, the presence of elastase, α2M, and alkaline phosphatase was measured in crevicular fluid collected from implants with and without clinical, radiographic, and microbiologic signs of peri-implantitis.[31] In comparison to clinically healthy implant sites, total amounts of all 3 substances were significantly higher in crevicular fluid collected around implants with peri-implantitis. In addition, such amounts were correlated with the clinical parameters, thus showing that these markers are associated not only with periodontal status but with peri-implant status as well. The same markers were used in the present investigation, the hypothesis being that implants with intracrevicular crown margins can remain stable, with surrounding tissues healthy over a long period of time. Thus, the longitudinal stability of 61 such implants was evaluated twice cross-sectionally by means of selected clinical, microbiologic, and biochemical parameters.

MATERIALS AND METHODS

Patient Population

The population for this longitudinal study consisted of 45 patients (22 women and 23 men, mean age 34.3 years) treated with a total of 61 maxillary anterior implants (ITI Dental Implant System, Institut Straumann, Waldenburg, Switzerland). Patients were randomly selected from the Schools of Dental Medicine of the University of Geneva and the University of Berne. The implants had been placed in accordance with a standardized surgical protocol[8] and were subsequently documented prospectively in

the frame of a multicenter study. All implant-supported single crown restorations had been in function for at least 1 year before the first examination (baseline). Patients were all in good health and had not received antibiotics during the 6 months prior to the beginning of the study.

Clinical Examination

The clinical evaluation was performed by 1 clinician and included measurement of probing pocket depth (PPD) and assessments of the Modified Plaque Index (mPl)[32] and of the Gingival Index (GI).[33] The presence or absence of suppuration (SI) and bleeding on probing (BOP) and the distance between implant shoulder and mucosal margin (DIM; Fig 1) were also recorded. Negative DIM values corresponded to a submucosal location of the implant shoulder. All measurements were performed at 4 sites around each implant and were carried out to the nearest mm using a Hu-Friedy PCP[12] periodontal probe (Immunity Steel Instruments, Chicago, IL). The stability of the implant was assessed by means of the Periotest electronic device (Siemens AG, Bensheim, Germany). All clinical evaluations were assessed at baseline (first examination) and again at a follow-up examination 3 years later.

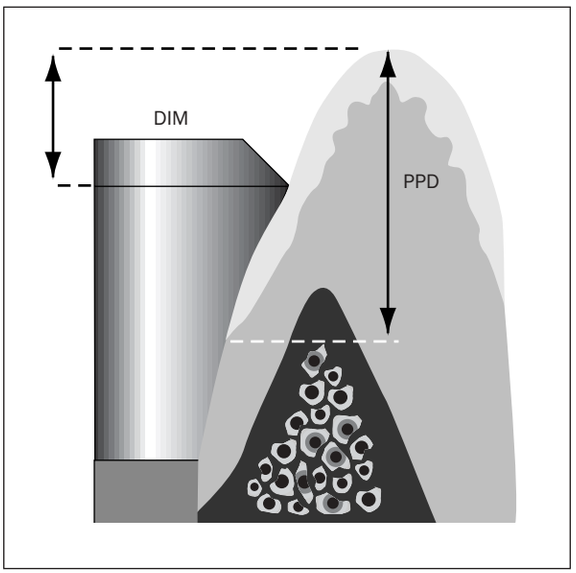

Fig 1 Depiction of the measurement of the distance between implant shoulder and mucosal margin (DIM) and the pocket probing depth (PPD). DIM values are negative if the implant shoulder is submucosal.

Crevicular Fluid Sampling

PICF was collected mesially and distally to each implant after the presence or absence of plaque was assessed and before registration of any other clinical parameters. The implant sites were isolated with cotton rolls and dried gently with compressed air. After 3 minutes, standardized paper strips (Periopaper, Pro Flow, Amityville, NY) were inserted into the sulci or pockets until slight resistance was felt and left in place for 15 seconds. The amount of fluid was then evaluated using the Periotron 8000 (Pro Flow). The strips were transferred immediately to plastic vials and stored at –20°C until the day of analysis.

Biochemical Analysis of Crevicular Fluid

One hundred microliters of phosphate-buffered saline (PBS), pH 7.2, were added to each sample. The tubes were vigorously shaken for 1 minute and then centrifuged at 2,000*g* for 5 minutes, with the strips kept at the collar of the tube to completely elute PICF components. After removal of the strip, the supernate was divided into 3 aliquots (1 for the determination of each biochemical compound). Elastase activity was determined using the fluorogenic substrate Meo-Suc-Ala-Ala-pro-Val/7-amino-4-methylcoumarin (MW 627.69) (Bachem, Bubendorf, Switzerland).[34,35]

The inhibitor α2M was determined by enzyme-linked immunosorbent assay.[36] The activity of alkaline phosphatase was measured by using p-nitrophenyl phosphate as substrate.[37]

Final results were expressed as total amounts per 15-second samples. Sites with levels below the limits of assay detectability were scored as 0 ng.

Microbiologic Sampling

Subgingival plaque samples were collected from the deepest site of each implant by using paper points. The plaque samples were placed in 100 µL of physiologic sterile solution and immediately examined by dark-field microscopy.[38] Immunofluorescence with specific monoclonal antibodies was used for the detection of the following pathogenic bacteria: *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Campylobacter rectus*.[39] Results were expressed as the percentage of sites positive to one of the target bacteria.

Statistical Analysis

Differences between implants at baseline (first examination) and 3 years later were tested for statistical significance using the Wilcoxon signed rank test. A value of $P < .01$ was required for statistical significance.

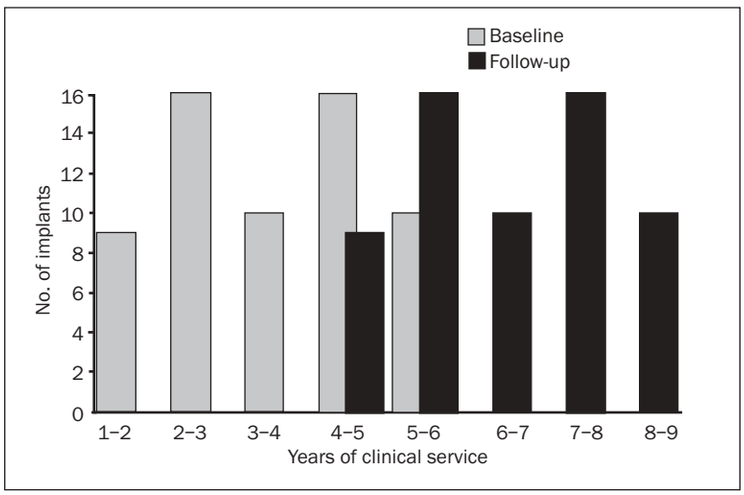

Fig 2 Distribution of implants at baseline and follow-up examination according to the number of years in function.

Table 1 Distribution of Implants According to Time in Function at Baseline

Group	Time in place (mo)	No. of implants	% of total
1	12–30	20	33
2	31–48	21	34
3	> 48	20	33

RESULTS

Implant Data and Clinical Results

The implants were divided into 3 groups according to the number of months they had been in function at baseline. As shown in Table 1, 33% of the implants belonged to the first group (12 to 30 months in function), 34% to the second group (31 to 48 months in function), and 33% to the third group (more than 48 months). The distribution of implants according to the number of years in function at baseline and at follow-up examination is shown in Fig 2. The implant features were as follows (n = 61):

- Implant position: 49 incisors, 12 canines.
- Implant type: 5 were hollow screws, 33 were hollow cylinders, 22 were solid screws, and 1 was a solid screw with reduced diameter.
- Implant length: 2 implants were 8 mm long, 23 were 10 mm long, 35 were 12 mm long, and 1 was 14 mm long.

Table 2 shows the clinical status of the implants at baseline and at follow-up examination, including PPD, percentage of sites with plaque accumulation or mucosal inflammation, and BOP. Also in Table 2, the mean DIM values at the mesiodistal (M-D) and vestibular-oral sites (V-O), as well as the mean Periotest values, are given for the 2 time points.

At baseline examination, a mean pocket probing depth of 4.2 ± 1.4 mm was found, and 30.3% of the sites revealed an mPI that was greater than zero. The majority of sites in which plaque was present showed an mPl of 1. With respect to the status of peri-implant mucosal health or inflammation, 40.9% of the sites were labeled with a GI greater than zero, with the majority corresponding to a GI of 1. BOP was detected in 49.6% of the sites, mostly in the form of a discrete blood point at the place where the periodontal probe had been applied. The DIM values, with means of –3.3 ± 1.5 mm interproximally and –1.5 ± 1.1 mm orofacially, clearly documented the submucosal implant shoulder location.

Finally, a mean Periotest value of –4.0 ± 3.4 confirmed the stability and osseointegration status of the implants. No statistically significant differences between baseline and follow-up examination were observed among the average clinical parameters, except for PPD and DIM, whose mean values increased slightly during the observation interval (Table 2).

Microbiologic Results

Dark-field microscopy revealed the presence of coccal bacteria and nonmotile rods in all peri-implant sulci at both the baseline and follow-up examinations. However, 23% of the implants at baseline and 29% at the 3-year examination showed the presence of spirochetes (Table 3). Table

Table 2 Clinical Data at Baseline and Follow-up Examinations (n = 61 implants)

Parameter	Baseline	Follow-up	*P*
PPD (mean ± SD)	4.2 ± 1.4 mm	4.6 ± 1.4 mm	.001
% of sites with			
mPI > 0	30.3	32.7	NS
GI > 0	40.9	39.3	NS
BOP = 1	49.6	39.3	NS
DIM value (mean ± SD)			
Mesiodistal	–3.3 ± 1.5 mm	–3.6 ± 1.4 mm	.001
Vestibular-oral	–1.5 ± 1.1 mm	–1.7 ± 1.1 mm	.001
Periotest value (mean ± SD)	–4.0 ± 3.4	–4.6 ± 2.9	NS

PPD = probing pocket depth; mPI = Modified Plaque Index; GI = Gingival Index; BOP = bleeding on probing; DIM = distance between implant shoulder and mucosal margin (negative value in case of submucosal implant shoulder); NS = not statistically significant.

Table 3 No. (%) of Samples Testing Positive for 4 Different Cell Morphotypes in the Dark-field Microscope (n = 61 implants)

Cell morphotype	Baseline	Follow-up
Cocci	61 (100%)	61 (100%)
Rods	61 (100%)	61 (100%)
Motile rods	7 (11%)	4 (6.5%)
Spirochetes	14 (23%)	18 (29.5%)

Table 4 No. (%) of Samples Testing Positive for Selected Bacterial Species in a total of 61 Samples

Species	Baseline	Follow-up
A actinomycetemcomitans	0 (0%)	2 (3%)
B forsythus	28 (46%)	33 (54%)
P gingivalis	9 (15%)	11 (18%)
P intermedia	28 (46%)	44 (72%)
C rectus	12 (20%)	17 (28%)

Table 5 Total Amounts (Mean ± SD) of Elastase, α2-macroglobulin, and Alkaline Phosphate in Implants at Baseline and Follow-up Examinations

	Baseline	Follow-up	*P*
Elastase activity (ng/sample)	7.2 ± 14	8.3 ± 6.8	NS
α2-macroglobulin (ng/sample)	8.4 ± 22	4.1 ± 7.2	NS
Alkaline phosphatase (μU/sample)	298 ± 325	241 ± 133	NS

4 shows the percentage of implants that tested positive for each of the pathogenic bacteria at baseline and 3-year examinations. *P intermedia* and *B forsythus* were detected more frequently at both time points, followed by *C rectus* and *P gingivalis*. All bacterial species showed a tendency to increase at the follow-up examination. However, only *P intermedia* showed significant changes between baseline and the 3-year examination. The presence of *A actinomycetemcomitans* was revealed only in 1 patient at his 2 implant sites, at the 3-year follow-up examination.

Biochemical Analysis of PICF

Elastase and alkaline phosphatase were regularly recovered from all PICF samples. However, α2M was absent from almost 46% of samples collected at baseline and from 60% of the samples collected 3 years later. Table 5 shows the mean total amount (± SD) of each biochemical parameter at baseline and follow-up examinations. For all 3 substances, no significant differences at the level of $P \leq .01$ were found between the 2 time points.

Finally, when implants were divided according to time in function, most of the clinical data and all biochemical and microbiologic parameters showed no significant differences between baseline and follow-up examination (Tables 6 and 7; Fig 3). Only the PPD measurements (all groups) and the DIM data for group 3 revealed slight increases over time (Table 6).

DISCUSSION

In the present study, the effect of intracrevicular crown margins on long-term peri-implant tissue health and stability was investigated. Esthetic zone implants that had been in function for 1 to 6 years at the moment of baseline examination were re-evaluated 3 years later (time of function: 4 to 9 years) using selected clinical, microbiologic, and biochemical parameters.

Table 6 Clinical Data of the 3 Groups of Implants at Baseline and Follow-up Examinations

Parameter	Baseline	Follow-up	*P*
PPD (mean ± SD)			
Group 1	4.5 ± 1.5 mm	4.9 ± 1.5 mm	.003
Group 2	4.1 ± 1.3 mm	4.5 ± 1.3 mm	.001
Group 3	3.9 ± 1.2 mm	4.5 ± 1.3 mm	.001
% of sites with			
mPI > 0			
Group 1	23	27	NS
Group 2	35	35	NS
Group 3	31	35	NS
GI > 0			
Group 1	31	46	NS
Group 2	47	42	NS
Group 3	44	30	NS
BOP = 1			
Group 1	49	46	NS
Group 2	47	42	NS
Group 3	54	30	NS
DIM value (mean ± SD) (M-D)			
Group 1	–3.5 ± 1.6 mm	–3.9 ± 1.7 mm	NS
Group 2	–3.4 ± 1.5 mm	–3.4 ± 1.3 mm	NS
Group 3	–3.1 ± 1.4 mm	–3.5 ± 1.3 mm	.002
DIM value (mean ± SD) (V-O)			
Group 1	–1.6 ± 1.1 mm	–1.8 ± 1.1 mm	NS
Group 2	–1.6 + 0.9 mm	–1.8 ± 1.2 mm	NS
Group 3	–1.3 ± 1.0 mm	–1.6 ± 1.2 mm	.008
Periotest value (mean ± SD)			
Group 1	–3.7 ± 3.5	–3.6 ± 3.3	NS
Group 2	–5.2 + 1.7	–5.6 ± 1.9	NS
Group 3	–3.1 ± 4.2	–4.5 ± 3.2	NS

Group 1: 12–30 months in function at baseline/48–66 months at follow-up (n = 20); group 2: 31–48 months in function at baseline/67–84 months at follow-up (n = 21); group 3: > 48 months in function at baseline/> 84 months at follow-up (n = 20).
PPD = probing pocket depth; mPI = Modified Plaque Index; GI = Gingival Index; BOP = bleeding on probing; DIM = distance between implant shoulder and mucosal margin; M-D = mesiodistal; V-O = vestibular-oral; NS = not statistically significant.

Table 7 Biochemical Data (Mean ± SD) of the 3 Groups of Implants at Baseline and Follow-up Examinations

Parameter	Baseline	Follow-up	*P*
Elastase activity (ng/sample)			
Group 1	5.1 ± 8.2	10.0 ± 6.8	NS
Group 2	6.1 ± 12.4	8.1 ± 7.4	NS
Group 3	10.5 ± 19	6.8 ± 5.8	NS
α2-macroglobulin (ng/sample)			
Group 1	11.0 ± 30	4.5 ± 5.8	NS
Group 2	4.5 ± 6.7	4.1 ± 6.7	NS
Group 3	9.8 ± 22	3.7 ± 8.9	NS
Alkaline phosphatase (μU/sample)			
Group 1	219 ± 161	233 ± 118	NS
Group 2	295 ± 279	265 ± 163	NS
Group 3	381 ± 450	224 ± 109	NS

Group 1: 12–30 months in function at baseline/48–66 months at follow-up (n = 20); group 2: 31–48 months in function at baseline/67–84 months at follow-up (n = 21); group 3: > 48 months in function at baseline/> 84 months at follow-up (n = 20).

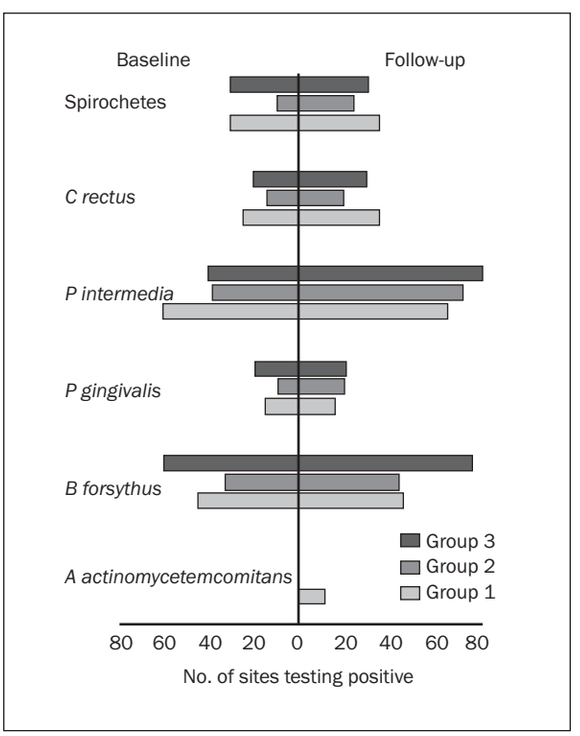

Fig 3 *(Right)* Percentage of implant sites positive for selected bacteria in the 3 groups of implants at baseline and follow-up examination. Group 1: 12 to 30 months in function at baseline/48–66 months at follow-up (n = 20); group 2: 31–48 months in function at baseline/67–84 months at follow-up (n = 21); group 3: more than 48 months in function at baseline/more than 84 months at follow-up (n = 20).

The monitoring of PPD, mPl, BOP, GI, DIM, and Periotest values permitted, on the one hand, the determination of the overall clinical status associated with anterior maxillary implants supporting fixed single crown restorations with intracrevicular margins, and on the other hand, the identification of any relevant changes between baseline and follow-up examination. The baseline data clearly demonstrated a clinically healthy status of the peri-implant tissues. More specifically, a mean PPD of 4.2 ± 1.4 mm is what one would expect to find around 2-part ITI implants placed significantly deeper for esthetic reasons, when compared to a standard, more superficial, transmucosal surgical protocol.[10,40–45] This parameter had a slight tendency to increase between baseline and follow-up (4.6 ± 1.4 mm) examinations, regardless of the time period the implants were in function (4 to 9 years) (Table 6). When it came to the presence or absence of clinically detectable plaque accumulation, mean values of 30.3% (baseline) and 32.7% (follow-up) were found, essentially corresponding to an mPl of 1. This parameter reflected a high overall level of plaque control, consistent for the 3 subgroups, and stable throughout the entire observation period. As far as GI and BOP were concerned, a low degree of inflammation of the peri-implant mucosa was observed, with a slight, but statistically not significant, tendency to decrease over time (Tables 2 and 6), again confirming favorable peri-implant conditions.

Probably the most critical parameter from a purely esthetic point of view is the DIM value, particularly on the labial aspect of the maxillary anterior implants investigated in this study.[9,10,43] A mean value of –1.5 ± 1.1 mm was found at baseline examination, and a slight increase (–1.7 ± 1.1 mm) was seen at follow-up. This indicates that the risk of exposure of the implant-to-crown interface or margin can be considered low. These findings corroborate recently published data.[43]

Finally, the consistently negative Periotest scores confirm the stability and osseointegration of the implants examined. Based on all of the previously mentioned clinical parameters, favorable peri-implant tissue conditions were consistently encountered and remained stable throughout the entire observation period of this study.

It is well established that the microbiota around stable versus failing implants share the same patterns as those around healthy versus diseased natural teeth. Specifically, microbiologic observations have identified 5 pathogenic bacteria known to be associated with periodontal disease and peri-implantitis.[46,47] The intracrevicular percentages of these bacteria were also tested by immunofluorescence in combination with dark-field microscopy. At baseline and 3-year follow-up examinations, the predominant morphotype was coccoid cells, and only very low levels of spirochetes and anaerobic motile rods were observed in some sites. Similarly, immunofluorescence revealed either the absence or only low levels of 1 of the 5 bacterial species at both baseline and follow-up examinations. Several authors consider the placement of intracrevicular crown margins to represent an etiologic factor for gingival/periodontal inflammation[48,49] and consequently for peri-implant inflammation. To date, only 1 preliminary study has aimed to determine the levels of *P gingivalis*, *P intermedia*, and *A actinomycetemcomitans* in the mucosal sulcus of esthetic implants[50] and concluded that none of the sulci sampled contained detectable levels of the 3 bacteria. However, this was a cross-sectional study with only moderate population size. Plaque monitoring may only have a diagnostic and/or prognostic value if it is followed longitudinally. In the present study, the low level of sulcular colonization by the bacterial species tested was sustained for 3 years, and no significant shifts in the composition of the microbiota were observed with time.

In recent years, the local host response in the context of periodontitis has been studied by biochemical analysis of GCF, and many host inflammatory and immune mediators have been identified as potential diagnostic or prognostic markers of periodontal destruction.[51,52] Similarly, biochemical markers in PICF have been identified in an effort to determine disease activity around implants at early stages of the pathologic process, thus allowing intervention before substantial amounts of bone are lost. Several molecules have been shown to be involved in the inflammatory response and tissue damage in peri-implantitis, such as IL-1β,[19,20] IL-6,[53] myeloperoxidase, β-glucuronidase, and prostaglandin E_2.[53,54]

Recently, elastase, α2M, and alkaline phosphatase have been shown to be significantly elevated in the PICF of implants with peri-implantitis, as compared to healthy implants.[31] The same parameters were used in the present investigation to study the "stability" of the peri-implant tissues around esthetic implants. The hypothesis was that a low level of these markers during the 3-year observation period would indicate a stable and healthy situation, whereas increased levels at the 3-year examination might indicate an active site. In the present study, similar amounts of elastase, α2M, and alkaline phosphatase were found at both baseline and follow-up examinations, and this independently from the number of years that the implants were in place.

Because of the inability to measure the extremely small quantities of PICF that were recovered from all implants, the levels of the biochemical compounds have been reported as total amounts per 15-second sample, as an alternative to concentrations. This is in accordance with the findings of several authors,[37,55,56] which suggested that total amounts of crevicular fluid components per site reflect better disease activity rather than concentrations.

SUMMARY

Based on these clinical, microbiologic, and biochemical data, and within the limits of this study, it was concluded that in patients with appropriate oral hygiene, implant-supported anterior maxillary crowns with intracrevicular margins are not predisposed to unfavorable peri-implant host and microbial responses. In particular, overall healthy and stable peri-implant tissue conditions, a paramount criterion when it comes to esthetic implant crowns, were consistently encountered and maintained longitudinally.

ACKNOWLEDGMENTS

The authors wish to thank PD Dr R. Gmür, Institute of Oral Microbiology and General Immunology, University of Zürich, Switzerland, for kindly providing the monoclonal antibodies for the detection of the bacterial target organisms. We would also like to thank Dr Ch. Balsiger, Senior Lecturer, Department of Oral Surgery and Stomatology, University of Bern, Switzerland, for his assistance during the clinical examination, as well as Prof P. Baehni, Department of Preventive Dentistry, University of Geneva, for generously providing the necessary equipment for the microbiologic analyses.

REFERENCES

1. Brånemark P-I, Hansson BO, Adell R, et al. Osseointegrated implants in the treatment of edentulous jaws: Experience from a 10-year period. Scand J Plast Reconstr Surg 1977;16: 1–132.
2. Adell R, Lekholm U, Rockler B, Brånemark P-I. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg 1981;10:387–416.
3. Babbush CA, Kent JN, Misiek DL. Titanium plasma-sprayed (TPS) screw implants for the reconstruction of the edentulous mandible. J Oral Maxillofac Surg 1986;44: 274–282.
4. Buser D, Weber HP, Lang NP. Tissue integration of non-submerged implants. 1-year results of a prospective study with 100 ITI hollow-cylinder and hollow-screw implants. Clin Oral Implants Res 1990;1:33–40.
5. Mericske-Stern R, Steinlin-Schaffner T, Marti P, Geering AH. Peri-implant mucosal aspects of ITI implants supporting overdentures. A five-year longitudinal study. Clin Oral Implants Res 1994;5:9–18.
6. Buser D, Mericske-Stern R, Bernard JP, et al. Long-term evaluation of non-submerged ITI implants. Part I: 8-year life table analysis of a prospective multi-center study with 2359 implants. Clin Oral Implants Res 1997;8:161–172.
7. Lindh T, Gunne J, Tillberg A, Molin M. A meta-analysis of implants in partial edentulism. Clin Oral Implants Res 1998; 9:80–90.
8. Buser D, von Arx T. Surgical procedures in partially edentulous patients with ITI implants. Clin Oral Implants Res 2000;11(suppl):83–100.
9. Belser UC, Buser D, Hess D, Schmid B, Bernard JP, Lang NP. Esthetic implant restorations in partially edentulous patients—A critical appraisal. Periodontol 2000 1998;17: 132–150.
10. Belser UC, Mericske-Stern R, Bernard JP, Taylor T. Prosthetic management of the partially dentate patient with fixed implant restorations. Clin Oral Implants Res 2000;11(suppl): 126–145.
11. Apse P, Ellen RP, Overall CM, Zarb GA. Microbiota and crevicular fluid collagenase activity in the osseointegrated dental implant sulcus: A comparison of sites in edentulous and partially edentulous patients. J Periodont Res 1989;24: 98–105.
12. Listgarten MA, Lang NP, Schroeder HE, Schroeder A. Periodontal tissues and their counterparts around endosseous implants. Clin Oral Implants Res 1991;2:1–19.
13. Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liéjenberg B, Thomsen P. The soft tissue barrier at implants and teeth. Clin Oral Implants Res 1991;2:81–90.
14. Buser D, Weber HP, Donath K, Fiorellini JP, Paquette DW, Williams RC. Soft tissue reactions to non-submerged unloaded titanium implants in beagle dogs. J Periodontol 1992;63;225–235.
15. Tonetti MS, Schmid J, Hämmerle CH, Lang NP. Intraepithelial antigen-presenting cells in the keratinized mucosa around teeth and osseointegrated implants. Clin Oral Implants Res 1993;4:177–180.
16. Last KS, Cawood JI, Howell RA, Embery G. Monitoring of Tübingen endosseous dental implants by glycosaminoglycans analysis of gingival crevicular fluid. Int J Oral Maxillofac Implants 1991;6:42–49.
17. Last KS, Smith S, Pender N. Monitoring of IMZ titanium endosseous dental implants by glycosaminoglycans analysis of peri-implant sulcus fluid. Int J Oral Maxillofac Implants 1995;10:58–65.
18. Eley BM, Cox SW, Watson RM. Protease activities in peri-implant sulcus fluid from patients with permucosal osseointegrated dental implants. Correlation with clinical parameters. Clin Oral Implants Res 1991;2:62–70.
19. Kao RT, Curtis DA, Richards DW, Preble J. Increased Interleukin-1β in the crevicular fluid of diseased implants. Int J Oral Maxillofac Implants 1995;10:696–701.
20. Panagakos FS, Aboyoussef H, Dondero R, Jandinski JJ. Detection and measurement of inflammatory cytokines in implant crevicular fluid: A pilot study. Int J Oral Maxillofac Implants 1996;11:794–799.
21. Page RC. Host response tests for diagnosing periodontal diseases. J Periodontol 1992;63:356–366.
22. Janoff A. Elastase in tissue injury. Ann Rev Med 1985;36: 207–216.

23. Gustafsson A, Asman B, Bergrstrom K. Elastase and lactoferrin in gingival crevicular fluid: Possible indicators of a granulocyte-associated specific host response. J Periodont Res 1994;29:276–282.
24. Ingman T, Sorsa T, Kangaspunta P, Konttinen YT. Elastase and α1-proteinase inhibitor in gingival crevicular fluid and gingival tissue in adult and juvenile periodontitis. J Periodontol 1994;5:702–709.
25. Palcanis KG, Larjava IK, Wells BR, Suggs KA, Landis JR, Chadwick DE, Jeffcoat MK. Elastase as an indicator of periodontal disease progression. J Periodontol 1992;63:237–242.
26. Armitage GC, Jeffcoat MK, Cadwick DE, et al. Longitudinal evaluation of elastase as a marker for the progression of periodontitis. J Periodontol 1994;65:120–128.
27. Kennett CN, Cox SW, Eley BM. Localization of active and inactive elastase, α-1-proteinase inhibitor, and α-2-macroglobulin in human gingiva. J Dent Res 1995;74:667–674.
28. Ichimaru E, Imura K, Hara Y, Kato Y, Kato I. Cystatin activity in gingival crevicular fluid from periodontal disease patients measured by a new quantitative analysis method. J Periodont Res 1992;27:119–125.
29. Nakashima K, Giannopoulou C, Andersen E, et al. A longitudinal study of various crevicular fluid components as markers of periodontal disease activity. J Clin Periodontol 1996;23:832–838.
30. Chapple ILC, Garner I, Saxby MS, Moscrop H, Matthews JB. Prediction and diagnosis of attachment loss by enhanced chemiluminescent assay of crevicular fluid alkaline phosphatase levels. J Clin Periodontol 1999;26:190–198.
31. Plagnat D, Giannopoulou C, Carrel A, Bernard J-P, Mombelli A, Belser U. Elastase, a2-macroglobulin and alkaline phosphatase in crevicular fluid from implants with and without periimplantitis. Clin Oral Implants Res 2002;13: 227–233.
32. Mombelli A, Lang NP. Clinical parameters for the evaluation of dental implants. Periodontology 2000 1994;4:81–86.
33. Löe H. The gingival index, the plaque index and the retention index systems. J Periodontol 1967;38:610–616.
34. Baici A. Interaction of human leukocyte elastase with soluble and insoluble protein substrates. A practical kinetic approach. Biochim Biophys Acta 1990;1040:355–364.
35. Castillo M, Nakajima K, Zimmermann M, Powers J. Sensitive substrates for human leukocyte and porcine pancreatic elastase: A study of the merits of various chromophoric and fluorogenic leaving groups in assays for serine proteases. Ann Biochem 1979;99:53–64.
36. Giannopoulou C, Andersen E, Demeurisse C, Cimasoni G. Neutrophil elastase and its inhibitors in human gingival crevicular fluid during experimental gingivitis. J Dent Res 1992;71:359-363.
37. Nakashima K, Roehrich N, Cimasoni G. Osteocalcin, prostaglandine E2 and alkaline phosphatase in gingival crevicular fluid: Their relations to periodontal status. J Clin Periodontol 1994;21:327-333.
38. Listgarten MA, Hellden L. Relative distribution of bacteria at clinically healthy and periodontally diseased sites in humans. J Clin Periodontol 1978;5:115-132.
39. Gmür R, Strub JR, Guggenheim B. Prevalence of *Bacteroides forsythus* and *Bacteroides gingivalis* in subgingival plaque of prosthodontically treated patients on short recall. J Periodontal Res 1989;24:113–120.
40. Berglundh T, Lindhe J, Johnson K, Ericsson I. The topography of the vascular systems in the periodontal and periimplant tissues in the dog. J Clin Periodontol 1994;21: 189–193.
41. Cochran DL. The scientific basis for and clinical experiences with the ITI Dental Implant System: A consensus report. Clin Oral Implants Res 2000;11(suppl):33–58.
42. Gotfredsen K, Hjørting-Hansen E, Budtz-Jørgensen E. Clinical and radiographical evaluation of submerged and nonsubmerged implants in monkeys. Int J Prosthodont 1990;3:463–469.
43. Grunder U. Stability of the mucosal topography around single-tooth implants and adjacent teeth: 1 year results. Int J Periodontics Restorative Dent 2000;20:11–17.
44. Hämmerle C, Brägger U, Bürgin W, Lang NP. The effect of subcrestal placement of the polished surface of ITI implants on marginal soft and hard tissues. Clin Oral Implants Res 1996;7:111–119.
45. Hermann JS, Cochran DL, Nummikoski PV, Buser D. Crestal bone changes around titanium implants. A histometric evaluation of unloaded nonsubmerged and submerged implants in the canine mandible. J Periodontol 1997;68: 1117–1130.
46. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal disease. Periodontology 2000 1994; 5:78–111.
47. Mombelli A, van Oosten MAC, Schurch E, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol 1987;2: 145–151.
48. Silness J. Periodontal conditions in patients treated with dental bridges. J Periodontal Res 1979;5:665–692.
49. App GR. Effect of silicate, amalgam and cast gold in gingiva. J Prosthet Dent 1961;11:522–532.
50. Kancyper SG, Koka S. The influence of intracrevicular crown margins on gingival health: Preliminary findings. J Prosthet Dent 2001;85:461–465.
51. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. J Periodontal Res 1993;28: 500–510.
52. Genco RJ. Host responses in periodontal diseases: Current concepts. J Periodontol 1992;63:338–355.
53. Salcetti JM, Moriarty JD, Cooper LF, et al. The clinical, microbial and host response characteristics of the failing implant. Int J Oral Maxillofac Implants 1997;12:32–42.
54. Boutros SM, Michalowicz BS, Smith QT, Aeppli DM. Crevicular fluid enzymes from endosseous dental implants and natural teeth. Int J Oral Maxillofac Implants 1996;11: 322–330.
55. Eley BM, Cox SW. Cathepsin B/L-, elastase-, tryptase-, trypsin- and dipeptidyl peptidase IV activities in gingival crevicular fluid: Correlation with clinical parameters in untreated chronic periodontitis patients. J Periodontal Res 1992;27:62–69.
56. Smith QT, Au GS, Freese PL, Osborn JB, Stoltenberg JL. Five parameters of gingival crevicular fluid from eight surfaces in periodontal health and disease. J Periodontal Res 1992;27:466–475.