



**ISIP**  
Italian Society of  
Periodontology

## Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans

Studio clinico comparato sulla reversibilità della mucosite peri-implantare e della gengivite sperimentali

Aglietta M., Eick S., Sculean A., Salvi G.E.

*Department of Periodontology, School of Dental Medicine, University of Bern, Bern, Switzerland*

PROCEEDINGS BOOK RESEARCH SESSION "HENRY M. GOLDMAN PRIZE" 2011 – ATTI DELLA SESSIONE DI RICERCA "PREMIO H.M. GOLDMAN" 2011

### Summary

Clinical, biochemical and microbiological parameters were monitored during a 3-week period of undisturbed plaque accumulation and, subsequently, a 3-week period of plaque control around implants and teeth. The reversibility of gingival and mucosal inflammation, experimentally elicited by plaque accumulation, was demonstrated.

### Riassunto

Parametri clinici, biochimici e microbiologici sono stati valutati intorno a denti ed impianti durante 3 settimane di accumulo indisturbato di placca e successive 3 settimane di controllo di placca ottimale. La reversibilità dell'infiammazione nei tessuti molli indotta dalla placca batterica intorno a denti ed impianti è stata dimostrata.

### Introduction

Peri-implant diseases represent a collective term for chronic inflammatory processes in the soft and hard tissues surrounding an oral implant. Peri-implant mucositis was defined as an inflammatory process in the soft tissues surrounding an implant, whereas peri-implantitis is characterized by additional loss of peri-implant bone.

Experimental studies in man and animal models provided evidence that gingivitis and periodontitis are dependent upon the presence of a bacterial biofilm on the tooth surfaces and the adjacent gingival margins (Løe et al. 1965). Similarly, findings from animal (Lindhe et al. 1992) and human studies (Pontoriero et al. 1994, Zitzmann et al. 2001) demonstrated clinically and histologically that a comparable reaction to an experimental biofilm accumulation could be obtained in the soft tissues adjacent to titanium oral implants.

No attempt, however, was made so far to evaluate and compare the healing sequence (i.e. resolution of inflammation) after experimental undisturbed plaque accumulation around teeth and titanium oral implants. Therefore, the aims of this clinical study were: i) to elucidate the clinical, microbiological and host-derived factors involved in the pathogenesis of experimental gingival/mucosal inflammation and ii) to compare the sequence of resolution of inflammation around teeth and implants after reinstatement of optimal mechanical plaque control.

### Material and Methods

The study was designed as a controlled 21-day experimental gingivitis/mucositis trial followed by a period of resolution of 21 days. The protocol was approved by the Ethical Committee of the Canton of Bern, CH (KEK-Gesuchs-Nr.: 133/08).

**Subject selection.** Inclusion criteria: 1i) age  $\geq$  18 years; 2i) healthy systemic conditions; 3i) cigarette smoking  $\leq$  5 cigarettes/day; 4i) healthy or treated periodontal conditions; 5i) full-mouth plaque score (FMPS)  $\leq$  15% at baseline; 6i) full-mouth bleeding score (FMBS)  $\leq$  15% at baseline; 7i) presence of a gap in the mandibular molar or premolar region restored with an implant-supported single-unit crown; 8i) presence of a mandibular contralateral premolar or molar; 9i) width of keratinized tissue  $\geq$  2 mm around experimental teeth and implants;

Exclusion criteria: 1e) control teeth with subgingival restoration margins; 2e) implants with a history of peri-implantitis; 3e) cigarette smoking  $>$  5 cigarettes/day; 4e) untreated periodontal conditions; 5e) full-mouth plaque score (FMPS)  $>$

15% at Baseline; 6e) full-mouth bleeding score (FMBS) > 15% at Baseline; 7e) pregnant or lactating females; 8e) width of keratinized tissue < 2 mm around experimental teeth and implants; 9e) implants supporting removable dental prostheses (RDP); 10e) untreated caries lesions; 11e) use of systemic antibiotics in the past 3 months; 12e) use of systemic antibiotics for endocarditis prophylaxis; 13e) patients chronically treated (i.e. two weeks or more) with any medication known to affect the periodontal status (e.g. phenytoin, calcium antagonists, cyclosporin, coumadin and non-steroidal anti-inflammatory drugs) within one month of the baseline examination; 14e) radiation therapy in the head and neck area; 15e) HIV, TB, hepatitis or other infectious diseases; 16e) drug and alcohol abuse;

*Clinical assessments and procedures.* After a prophylaxis procedure (instruction in optimal oral hygiene, scaling and polishing of the entire dentition) the subjects were monitored for 4 weeks. The ability to perform proper plaque control (FMPS and FMBS  $\leq$ 15%) was assessed before entering the experimental phase.

The subjects were then asked to abstain from oral hygiene practices in the posterior area of the mandible (47-43, 33-37) for a period of 21 days, following which all subjects resumed their optimal mechanical plaque control practices. At baseline and every 7 days for 6 weeks (e.g. T0, T7, T14, T21, T28, T35, T42), the following clinical parameters were assessed at 6 sites of each experimental unit:

- Plaque Index (PII; Silness&Løe 1964) and modified Plaque Index (mPII; Mombelli et al. 1987).
- Gingival Index (GI; Løe&Silness 1963) and modified Gingival Index (mGI).

The pocket probing depths (PPD) were assessed at 6 sites at T0, T21 and T42.

*Bacterial sampling and analysis.* Sterile paper points were inserted in the mesio-buccal site of each experimental unit (tooth/implant) for 30s. The samples were placed in separate Eppendorf tubes containing 0.15 ml TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) and 0.15 ml of 0.5 M NaOH was added. The counts of 40 species were determined in each plaque sample using a modification of the checkerboard DNA-DNA hybridization technique (Haffajee et al. 1997).

*Crevicular fluid sampling and analysis.* Crevicular fluid for determination of the host-derived biomarkers interleukin-1beta (IL-1 $\beta$ ) and matrix-metalloproteinase-8 (MMP-8) was collected by means of sterile paper strips (Periopaper, Oraflow Inc., Smithtown, NY, USA) inserted for 30s at the mesio-buccal sites at each experimental unit. Paper strips were stored at -80°C until assayed. One day before analysis, samples were eluted at 4°C overnight into 700 ml phosphate buffered saline (PBS) containing proteinase inhibitors (Sigma-Aldrich, St. Louis, MO, USA). After being centrifuged, the paper strips were removed and 100 ml aliquots of supernatant were used. The concentrations of MMP-8 and IL-1 $\beta$  were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Europe Ltd., Abingdon, UK).

*Statistical analysis.* The null hypothesis of no significant differences of the parameters assessed during an experimental 3-week period of undisturbed plaque accumulation and a 3-week period of resumed oral hygiene practices between teeth and implants was tested using the Wilcoxon rank sum test (statistical significance was set at  $\alpha = 0.05$ ).

## Results

All 15 subjects (8 males and 7 females) that entered into the study completed the final examination at Day 42. At Day 28, one subject did not show up for the examination.

*Clinical parameters.* At baseline, the median Plaque Index (PII) was 0.00 and 0.17 at implant and tooth sites, respectively (Table 1) ( $p < 0.05$ ). After one, two and three weeks of plaque accumulation, the median PII increased to 1.17, 1.33 and 1.33 at implants and to 1.33, 1.67 and 1.67 at teeth, respectively. The differences between the median PII at implant and tooth sites at all three observation periods were statistically significant ( $p < 0.02$ ).

At T28, T35 and T42, the median PII at implants reached 0.0, while it was at 0.33, 0.17 and 0.17 for the teeth. During this phase, median PII were not statistically significantly different at implants and teeth.

At T0, the median Gingival Index (GI; gingivitis/mucositis) was 0.17 and 0.00 for implants and teeth, respectively (difference implants – teeth  $p = 0.12$ ) (Table 1). At T7, T14 and T21, the median GI increased significantly to 1.33, 1.33 and 1.50 at implants and to 0.83, 1.00 and 1.00 at teeth, respectively. The differences between the two experimental units were statistically significant ( $p = 0.01$ ,  $p = 0.04$  and  $p = 0.02$ ). The median GI decreased significantly to 0.75, 0.50 and 0.50 at T28, T35 and T42 at implant sites, respectively. The corresponding mean GIs at teeth decreased to 0.83, 0.33 and 0.33. At 42 days, the difference between the median GI at implants and teeth was statistically significant ( $p = 0.04$ ).

Table 1. Plaque and gingival indexes at implants and teeth at different time-points of the study

Time point		T0	T7	T 14	T 21	T 28	T35	T 42	
Number of subjects		(n=15)	(n=15)	(n=15)	(N=15)	(n=14)	(n=15)	(n=15)	
PI	<b>Implants</b>								
	Minimum	0,000	0,170	0,500	0,830	0,000	0,000	0,000	
	25% Percentile	0,000	0,667	1,000	1,000	0,000	0,000	0,000	
	Median	0,000	1,167	1,333	1,333	0,000	0,000	0,000	
	75% Percentile	0,167	1,333	1,500	1,667	0,333	0,000	0,167	
	Maximum	0,170	2,000	2,170	2,000	1,170	0,170	0,330	
	<b>Teeth</b>								
	Minimum	0,000	0,330	1,000	1,000	0,000	0,000	0,000	
	25% Percentile	0,000	1,000	1,333	1,333	0,000	0,000	0,000	
	Median	0,167	1,333	1,667	1,667	0,333	1,667	1,667	
	75% Percentile	0,333	1,500	1,833	2,000	0,500	0,333	0,167	
	Maximum	0,670	2,000	2,170	2,170	0,830	0,500	0,330	
	<b>Statistics</b>								
	<i>Implants vs Teeth<sup>1</sup></i>	<b>0,046</b>	<b>0,016</b>	<b>0,018</b>	<b>0,003</b>	0,139	<b>0,041</b>	0,197	
	<b>Statistics Implants</b>								
<i>All T<sup>2</sup></i>	<b>&lt;0,001</b>								
<i>Vs. previous T<sup>1</sup></i>		<b>0,001</b>	<b>0,049</b>	0,389	0,001	0,058	0,059		
<i>Vs. T0<sup>1</sup></i>			<b>0,001</b>	<b>0,001</b>	0,121	0,655	0,102		
<b>Statistics Teeth</b>									
<i>All T<sup>2</sup></i>	<b>&lt;0,001</b>								
<i>Vs. previous T<sup>1</sup></i>		<b>0,001</b>	<b>0,011</b>	0,470	<b>0,001</b>	0,070	0,746		
<i>Vs. T0<sup>1</sup></i>			<b>0,001</b>	<b>0,001</b>	0,085	0,623	0,947		
GI	<b>Implants</b>								
	Minimum	0,000	0,000	0,670	1,000	0,170	0,170	0,00	
	25% Percentile	0,000	1,167	1,000	1,333	0,333	0,333	0,167	
	Median	0,167	1,333	1,333	1,500	0,750	0,500	0,500	
	75% Percentile	0,333	1,333	1,500	1,667	1,042	0,667	0,667	
	Maximum	0,500	1,830	2,000	2,000	1,330	1,500	1,000	
	<b>Teeth</b>								
	Minimum	0,000	0,330	0,670	0,500	0,170	0,170	0,000	
	25% Percentile	0,000	0,500	0,833	1,000	0,667	0,333	0,000	
	Median	0,000	0,833	1,000	1,000	0,833	0,333	0,333	
	75% Percentile	0,167	1,000	1,167	1,500	0,875	0,500	0,333	
	Maximum	0,330	1,170	1,330	1,830	1,000	0,670	0,500	
	<b>Statistics</b>								
	<i>Implants vs Teeth<sup>1</sup></i>	0,119	<b>0,010</b>	<b>0,039</b>	<b>0,016</b>	0,843	0,451	<b>0,032</b>	
	<b>Statistics Implants</b>								
<i>All T<sup>2</sup></i>	<b>&lt;0,001</b>								
<i>Vs. previous T<sup>1</sup></i>		<b>0,001</b>	0,200	<b>0,045</b>	<b>0,001</b>	<b>0,042</b>	0,698		
<i>Vs. T0<sup>1</sup></i>			<b>0,001</b>	<b>0,001</b>	<b>0,002</b>	<b>0,003</b>	<b>0,012</b>		
<b>Statistics Teeth</b>									
<i>All T<sup>2</sup></i>	<b>&lt;0,001</b>								
<i>Vs. previous T<sup>1</sup></i>		<b>0,001</b>	<b>0,037</b>	<b>0,017</b>	<b>0,005</b>	<b>0,002</b>	<b>0,005</b>		
<i>Vs. T0<sup>1</sup></i>			<b>0,001</b>	<b>0,001</b>	<b>0,001</b>	<b>0,001</b>	<b>0,029</b>		

<sup>1</sup> Wilcoxon test

<sup>2</sup> Friedman-Test

The median PPDs at implant sites were 3.00 mm at baseline, 2.83 mm at T21, and 2.83 mm at T42. At tooth sites, the corresponding values were 2.33 mm for all observation periods. These lower medians were statistically highly significant ( $p < 0.001$ ) (Table 2).

Table 2. Probing pocket depth at implants and teeth at T0, T21 and T42

Time point Number of subjects	T0 (n=15)	T21 (N=15)	T42 (n=15)
<b>Implants</b>			
Minimum	2,17	2,67	0,00
25% Percentile	2,67	2,67	2,67
Median	3,00	2,83	2,83
75% Percentile	3,33	3,33	3,33
Maximum	3,67	4,00	3,67
<b>Teeth</b>			
Minimum	1,67	1,67	1,50
25% Percentile	1,83	2,17	1,83
Median	2,33	2,33	2,33
75% Percentile	2,67	2,67	2,83
Maximum	3,00	2,83	2,83
<b>Statistics</b>			
<i>Implants vs Teeth<sup>1</sup></i>	<b>0,001</b>	<b>0,001</b>	<b>0,001</b>
<b>Statistics Implants</b>			
All T <sup>2</sup>	0,230	0,134	0,110
Vs. previous T <sup>1</sup>			0,823
Vs. T0 <sup>1</sup>			
<b>Statistics Teeth</b>			
All T <sup>2</sup>	0,537	0,342	0,235
Vs. previous T <sup>1</sup>			0,796
Vs. T0 <sup>1</sup>			

<sup>1</sup> Wilcoxonstest

<sup>2</sup> Friedman-Test

Table 3. Concentration of biomarkers MMP-8 and IL-1 $\beta$  in the crevicular fluid at different time-points of the study

Time point Number of subjects	T0 (n=15)	T7 (n=15)	T14 (n=15)	T21 (N=15)	T28 (N=14)	T35 (n=15)	T42 (n=15)
<b>MMP-8</b>							
<b>Implantat</b>							
Minimum	1,59	8,66	70,74	47,47	8,47	23,12	0,00
25% Percentile	32,14	185,60	413,40	795,00	158,78	296,00	402,66
Median	438,11	987,91	1761,73	1678,26	475,54	948,36	827,92
75% Percentile	1468,26	1836,26	2098,35	2460,66	1722,75	1887,82	1626,50
Maximum	2048,41	2774,49	3167,69	3163,49	2541,00	2649,05	2055,95
<b>Zahn</b>							
Minimum	1,33	0,00	13,72	61,00	18,89	0,00	0,00
25% Percentile	5,13	11,21	116,11	128,97	149,87	89,17	35,05
Median	71,70	302,58	506,85	883,59	282,39	308,42	158,31
75% Percentile	301,39	759,81	1007,86	1256,20	1384,23	1014,14	720,96
Maximum	1086,55	2301,09	2691,20	1932,31	2347,81	1437,11	2221,12
<b>Statistics</b>							
<i>Implants vs Teeth<sup>1</sup></i>	<b>0,012</b>	<b>0,031</b>	<b>0,023</b>	<b>0,008</b>	0,778	<b>0,031</b>	<b>0,020</b>
<b>Statistics Implants</b>							
All T <sup>2</sup>	<b>0,002</b>						
Vs. previous T <sup>1</sup>		0,088	0,078	1,000	<b>0,009</b>	0,510	0,125
Vs. T0 <sup>1</sup>			<b>0,001</b>	<b>0,012</b>	0,158	<b>0,020</b>	0,307
<b>Statistics Teeth</b>							
All T <sup>2</sup>	<b>0,029</b>						
Vs. previous T <sup>1</sup>		0,125	0,307	0,570	0,826	0,433	0,556
Vs. T0 <sup>1</sup>			<b>0,023</b>	<b>0,015</b>	<b>0,009</b>	0,191	0,496

IL-1 $\beta$	<b>Implants</b>							
	Minimum	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	25% Percentile	1,02	1,09	3,80	2,13	1,28	1,23	1,36
	Median	3,06	2,84	5,63	7,91	3,62	2,92	3,36
	75% Percentile	4,10	6,88	11,08	11,90	5,82	3,95	4,40
	Maximum	6,41	28,92	23,22	28,20	7,28	7,59	6,96
	<b>Teeth</b>							
	Minimum	0,00	0,00	0,00	1,57	0,00	0,00	0,00
	25% Percentile	0,00	1,98	1,43	2,99	1,28	1,29	1,29
	Median	2,13	3,21	4,56	4,18	2,13	2,48	2,13
	75% Percentile	2,84	5,94	8,07	10,91	3,45	4,63	4,86
	Maximum	4,86	13,32	21,20	16,79	5,48	6,65	9,04
	<b>Statistics</b>							
	Implants vs Teeth <sup>1</sup>	0,422	0,650	0,173	0,334	0,116	0,826	0,955
	<b>Statistics Implants</b>							
All T <sup>2</sup>	<b>0,005</b>							
Vs. previous T <sup>1</sup>		0,099	0,173	0,397	<b>0,035</b>	0,347	0,594	
Vs. T0 <sup>1</sup>			<b>0,005</b>	<b>0,006</b>	0,286	0,670	0,460	
<b>Statistics Teeth</b>								
All T <sup>2</sup>	<b>0,002</b>							
Vs. previous T <sup>1</sup>		0,006	0,300	0,776	<b>0,001</b>	0,925	0,975	
Vs. T0 <sup>1</sup>			<b>0,008</b>	<b>0,003</b>	0,300	0,281	0,460	

<sup>1</sup> Wilcoxon test

<sup>2</sup> Friedman-Test

In contrast, the medians of the IL-1 $\beta$  marker in the crevicular fluid at implant sites did not differ significantly at any observation periods from those observed at tooth sites (Table 3).

**Microbiological parameters.** The total DNA counts of the 40 species analyzed at teeth and implants did not differ at any observation periods throughout the study. At baseline, 73% of the subjects yielded at both experimental units  $\geq 10^5$  bacteria. This proportion increased to 80% after three weeks of undisturbed plaque accumulation and reassumed values of 47.7% and 60.0% of the subjects at T42.

*P. gingivalis* was only occasionally detected in high numbers (i.e.  $\geq 10^5$ ) after three weeks of abolished oral hygiene (Table 4). *T. forsythia* and *T. denticola* were isolated in a small proportion of subjects both at implant and tooth sites without any trends for increasing detection frequency during the period of abolished oral hygiene.

Among the orange complex species, only *Eikenella corrodens* showed a statistically significant increase in subjects with high *E. corrodens* counts during the period of abolished oral hygiene, irrespective of implant or tooth sites. This increase was reversed after one week of reinstatement of oral hygiene practices in both implant and tooth sites.

Table 4. Number of implants and teeth with  $\geq 10^5$  bacteria of the red complex (e.g. Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola).

	T0 (n=15)	T7 (n=15)	T14 (n=15)	T21 (n=15)	T28 (n=14)	T35 (n=15)	T42 (n=15)
<b>Implants</b>							
<i>P. gingivalis</i>	0	1	0	0	0	0	1
<i>T. forsythia</i>	5	5	5	5	5	7	5
<i>T. denticola</i>	3	7	6	5	6	7	4
<b>Teeth</b>							
<i>P. gingivalis</i>	0	0	0	1	0	0	0
<i>T. forsythia</i>	3	6	4	6	6	4	5
<i>T. denticola</i>	3	6	4	4	6	5	5

Figure 1. Boxplots of crevicular fluid levels (pg/site) of matrix metalloproteinase-8 (MMP-8) at implant sites during 21 days of experimental mucositis and 21 days of reinstated oral hygiene practices.

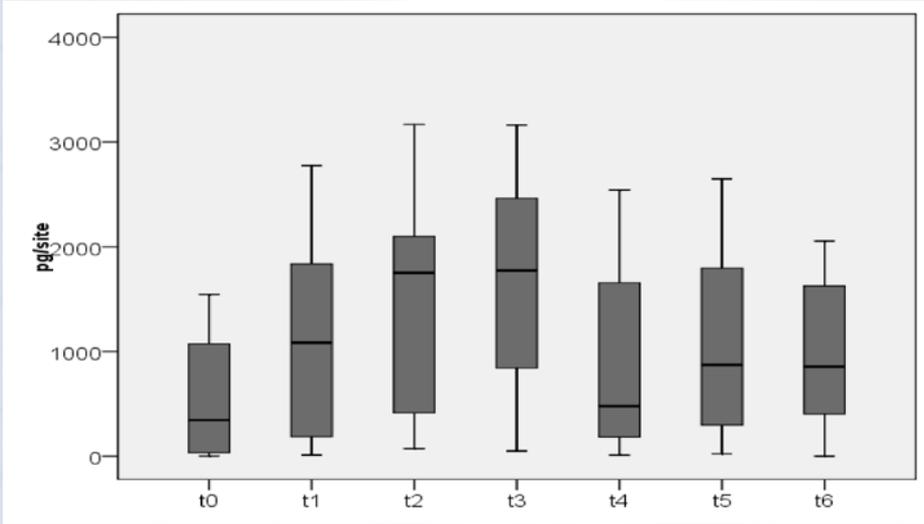
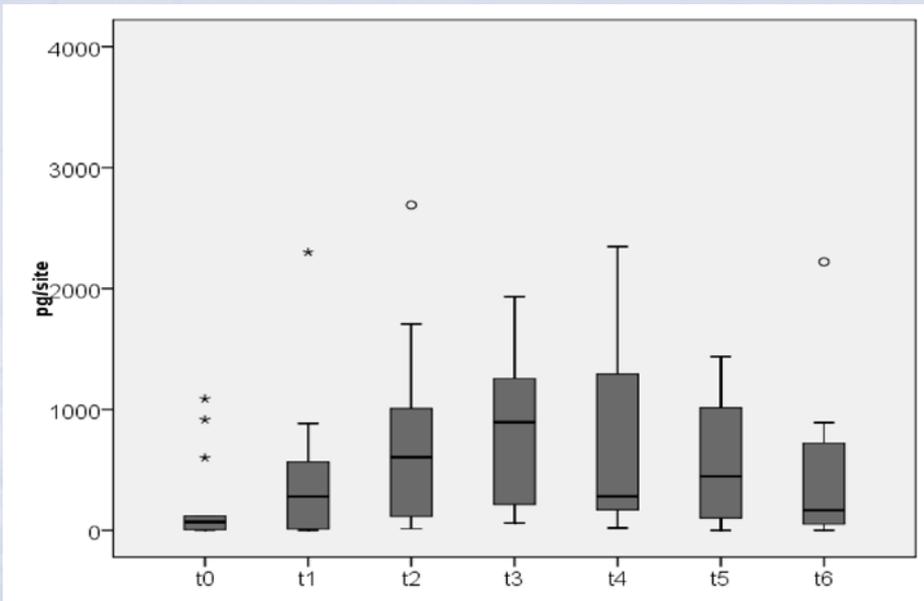


Figure 2. Boxplots of crevicular fluid levels (pg/site) of matrix metalloproteinase-8 (MMP-8) at tooth sites during 21 days of experimental gingivitis and 21 days of reinstated oral hygiene practices.



## Discussion

The present study showed that a comparable cause-effect relationship was established between the bacterial challenge and the host response around dental implants and natural teeth. The elimination of the biofilm resulted in an almost complete resolution of inflammation and the re-establishment of tissue health. Hence, the concept of prevention of gingivitis and periodontitis must apply to peri-implant diseases as well.

These results are in agreement with those of two controlled human studies addressing the effect of biofilm formation on the development of the inflammatory response at implant sites (Pontoriero et al. 1994, Zitzmann et al. 2001). Although

a kind of cause-and-effect relationship between the biofilm accumulation and the development of peri-implant mucositis was claimed in these two studies, a true cause-and-effect relationship depends on the establishment of reversibility to homeostasis of the model. In the present study, reversibility was established as identified by the decrease to pre-experimental levels of crevicular fluid markers such as MMP-8 and IL-1 .

Crevicular fluid levels of MMP-8 have been recognized as a marker of neutrophil function indicating an active phase of inflammation. In this respect, the significantly higher increase of MMP-8 at implant versus tooth sites is of special interest. Obviously, the bacterial challenge elicited a greater inflammatory response in the peri-implant mucosa than that encountered in the gingiva. This has already been shown in an animal experiment (Ericsson et al. 1992) where the volume of the inflammatory infiltrate was at least three times as large at implant compared to tooth sites within the same dogs after three months of experimental plaque accumulation.

Microbiologically, small differences across time and virtually no differences between implant and tooth sites were found. Putative periodontal pathogens were detected in a limited number of subjects. It has to be assumed that these pathogens do not represent the predominant microbiota associated with the development of experimental gingivitis/mucositis.

Although the PII differed significantly between implant and tooth sites at baseline, it has to be realized that the PII reached after the pre-experimental phase were extremely low. After three weeks of abolished oral hygiene, the PII at tooth sites was significantly elevated when compared to that of the implant sites. Again, this difference may not be clinically relevant, since the difference amounted to only 0.33. On the other hand, the increase of the GI at tooth sites was significantly lower than the one at implant sites indicating a more pronounced inflammatory response at implant sites. Moreover, during the three weeks of reinstated oral hygiene, the GI at implant sites dropped significantly less than that at tooth sites. Also, at both sites, pre-experimental levels of GI were not reached after 21 days of reinstated plaque control, indicating that the resolution of gingivitis/mucositis requires longer time of meticulous plaque control to meet the pre-experimental homeostasis.

In the present study, the PPD at the peri-implant units was consistently higher compared to that at dentogingival units at all observation times. As demonstrated before (Christensen et al. 1997), probe penetration with standardized forces results in values that average 0.5 mm more at implants compared to teeth.

In conclusion, three weeks of experimental plaque accumulation resulted in a reversible host response, e.g. gingivitis and peri-implant mucositis, at the biomarkers level, and a cause-and-effect relationship was confirmed between biofilm formation and gingivitis/ mucositis. However, a longer period of time is probably required to completely re-establish the initial homeostasis at the clinical level.

Peri-implant tissues seem to be more susceptible than gingival tissues to biofilm accumulation, displaying a more pronounced inflammatory response as well as a slower resolution of the inflammatory lesion.

## References

Christensen, M. M., Joss, A. & Lang, N. P. (1997) Reproducibility of automated periodontal probing around teeth and osseointegrated oral implants. *Clinical Oral Implants Research* 8: 455-464.

Ericsson, I., Berglundh, T., Marinello, C., Liljenberg, B. & Lindhe, J. (1992) Long-standing plaque and gingivitis at implants and teeth in the dog. *Clinical Oral Implants Research* 3: 99-103.

Haffajee, A.D., Cugini, M. A., Dibart, S., Smith, C., Kent, R.L. & Socransky, S.S. (1997) The effect of SRP on the clinical and microbiological parameters of periodontal diseases. *Journal of Clinical Periodontology* 24: 324-334.

Lindhe, J., Berglundh, T., Ericsson, I., Liljenberg, B. & Marinello C.(1992) Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. *Clinical Oral Implants Research* 3: 9-16.

Löe, H. & Silness, J. (1963) Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* 21: 533-551.

Löe, H., Theilade, E. & Börglum Jensen S. (1965) Experimental gingivitis in man. *Journal of Periodontology* 36, 5-15.

Mombelli, A., van Oosten, M.A.C., Schürch, E. & Lang, N.P. (1987) The microbiota associated with successful or failing

osseointegrated titanium implants. *Oral Microbiology and Immunology* 2: 145-151.

Pontoriero, R., Tonelli, M. P., Carnevale, G., Mombelli, A., Nyman, S. R. & Lang, N. P. (1994) Experimentally induced peri-implant mucositis. A clinical study in humans. *Clinical Oral Implants Research* 5: 254-259.

Silness, J. & Løe, H. (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal disease. *Acta Odontologica Scandinavica* 22, 121-135.

Zitzmann, N. U., Berglundh, T., Marinello, C. P. & Lindhe, J. (2001) Experimental peri-implant mucositis in man. *Journal of Clinical Periodontology* 28: 517-523.

