Int. J. Oral Maxillofac. Surg. 2019; 48: 554–559
https://doi.org/10.1016/j.ijom.2018.10.018, available online at https://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Clinical Paper
Dental Implants

Experimental peri-implant mucositis around titanium and zirconia implants in comparison to a natural tooth: part 1—host-derived immunological parameters

Kim Clever[1], Karl Andreas Schlegel[1], Heinz Kniha[2], Georg Conrads[3], Lothar Rink[4], Ali Modabber[5], Frank Hölzle[5], Kristian Kniha[5]

[1]Department of Oral and Cranio-Maxillofacial Surgery, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Erlangen, Germany; [2]Private Dental Clinic Drs. Kniha and Gahlert, Munich, Germany; [3]Division of Oral Microbiology and Immunology, Department of Operative Dentistry, Periodontology and Preventive Dentistry, University Hospital RWTH Aachen, Aachen, Germany; [4]Institute of Immunology, University Hospital RWTH Aachen, Aachen, Germany; [5]Department of Oral and Cranio-Maxillofacial Surgery, University Hospital RWTH Aachen, Aachen, Germany

Kim Clever, Karl Andreas Schlegel, Heinz Kniha, Georg Conrads, Lothar Rink, Ali Modabber, Frank HölzleKristian?Kniha: Experimental peri-implant mucositis around titanium and zirconia implants in comparison to a natural tooth: part 1—host-derived immunological parameters. Int. J. Oral Maxillofac. Surg. 2019; 48: 554–559.

Abstract. The purpose of this study was to assess host-derived parameters around dental zirconia and titanium implants and natural teeth during the occurrence of mucositis. After 4 weeks of perfect oral hygiene, 16 clinically profiled patients were asked to refrain from oral hygiene for 2 weeks, resulting in experimental plaque accumulation. This was followed by 4 weeks of perfect oral hygiene to reverse the inflammation. Immunological samples were analyzed for interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), and interleukin 1β (IL-1β). Immunological parameters were measured each week, starting at week 4 (session 2) and ending at week 10 (session 8). There were significant differences in IL-6 between the groups (zirconia vs. tooth and titanium vs. tooth), with unfavourable values for the tooth unit ($P < 0.05$). After reinstitution of oral hygiene, there was a significant increase in TNF-α values for the tooth but not for the zirconia and titanium implants. There were significant differences in IL-1β between the groups (zirconia vs. titanium and titanium vs. tooth), with higher IL-1β levels around titanium implants ($P < 0.05$). The soft tissue around titanium implants developed a stronger inflammatory response to experimental plaque accumulation in terms of IL-1β values, whereas the teeth presented an increase in IL-6 and TNF-α values.

Key words: experimental reversible mucositis; zirconia; host response; plaque accumulation; titanium; dental implant.

Accepted for publication 23 October 2018
Available online 5 November 2018

0901-5027/040554 + 06

The demand for dental implants, as well as the frequency of implantation, has risen to an all-time high[1–3]. The development of resistant oxide ceramics in dental implantology, such as alumina and zirconia, has provided new metal-free treatment options for patients and clinicians. In comparison to other ceramics, zirconia has shown superior biomechanical characteristics such as high fracture toughness and bending strength[4]. Additionally, patients are increasingly requesting highly aesthetic and long-term dental reconstructions. A recent study showed the simultaneous increase in cases of peri-implant disease, such as mucositis and peri-implantitis, which are factors affecting implant survival and success[5]. Of note, zirconia has been demonstrated to be very favourable for the formation of epithelial attachments and mucosal conditions[6], which are seen as important prerequisites for preventing peri-implant infections. However, science-based clinical results for commercially available zirconia dental implants are scarce.

The aim of this prospective cohort study was to evaluate and compare host-derived parameters around dental zirconia and titanium implants and natural teeth during the occurrence of experimental mucositis and subsequent recovery; this was achieved through the assessment of the immunological parameters interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), and interleukin 1β (IL-1β). The study patients performed optimal oral hygiene, including mechanical plaque control, before and after experimental plaque accumulation.

Materials and methods

Written informed consent was obtained from all study participants. Examinations and data collection were performed at one clinic, starting on January 13, 2015 and ending on December 15, 2015. A single examiner performed each assessment for clinical, immunological, and microbiological diagnosis during the entire study period. The study protocol was approved by the local ethics committee in accordance with the principles of the Declaration of Helsinki. Patients who had received one titanium implant and one zirconia implant were included in this prospective cohort study. This study was conducted in accordance with the STROBE statement (Strengthening the Reporting of Observational Studies in Epidemiology)[7].

Inclusion criteria were: (1) patients who voluntarily signed the informed consent and data protection consent form; (2) patients with healthy or treated periodontal conditions; (3) patients who had received both a titanium implant (Standard Plus, tissue level; Straumann AG, Basel, Switzerland) and a zirconia implant (PURE Ceramic, tissue level; Straumann AG); (4) implants in function for at least 1 year, with a maximum of 5 years; (5) implants and control teeth without any clinical or radiological signs of inflammation, such as bone loss, bleeding on probing, or pocket depth >5 mm.

Exclusion criteria were: systemic disease; current irradiation therapy; severe bruxism or clenching habits; patients not motivated to perform adequate oral hygiene; patients who smoked; physical or mental conditions that would interfere with the ability to perform adequate oral hygiene; age <18 years; pregnancy; patients treated chronically (i.e., for 2 weeks or more) with any medication known to affect periodontal status or the immune system (e.g., phenytoin, calcium antagonist, cyclosporine, coumadin, and non-steroidal anti-inflammatory drugs); untreated periodontal condition or implants with a history of peri-implantitis; use of systemic antibiotics within the past 3 months.

Prior to the experimental phase of the study, the patients were treated with oral prophylaxis and given instructions on perfect oral hygiene for both the upper and lower jaws. The patients were also instructed and asked to perform the best possible tooth cleaning twice a day for 4 weeks using the method described by Bass in 1954[8] (Table 1). The ability to perform proper plaque control was assessed for each patient before the experimental phase was commenced. At baseline, the deepest site around each unit (tooth and both implants) was chosen for later analysis. The patients were then asked to refrain from oral hygiene practices in the upper jaw for a period of 2 weeks. Subsequently, they were again treated with oral prophylaxis and given hygiene instructions. Furthermore, they again practiced optimal tooth cleaning for the next 4 weeks (Table 1). Parameter assessment was performed on one zirconia implant, one titanium implant, and one natural tooth. The same units in each patient were investigated throughout the study. All crevicular fluid samples were evaluated at a university clinic. Sterile rubber gloves and sterile instruments were used for sample assessment to avoid contamination of the samples.

Gingival/peri-implant mucosal crevicular fluid sampling and analysis

The deepest probing pocket around each implant/tooth, identified as the deepest site at the time of baseline measurements, was sampled using sterile paper points with coloured bands (29 mm, ISO 25, taper .02; VDW, Munich, Germany). At each session (session 2 at week 4 to session 8 at week 10), the samples were taken from the same unit site. Before assessment, the paper point was cut off at the lowest coloured band under sterile conditions (Fig. 1A). The gingival/peri-implant mucosal crevicular fluid was then sampled until it reached the second of the remaining coloured bands, following which the defined tip between the two band marks was cut off (Fig. 1B, C). Four of these tips were collected in one tube filled with 350 μl phosphate buffered saline (PBS; Sigma-Aldrich, St. Louis, USA) + 10% foetal calf serum (FCS; PAA Laboratories, Germany) (Eppendorf tubes, 1.5 ml; VWR International GmbH, Langenfeld, Germany); these were stored at −80 °C. On each assessment day, a control sample was prepared using four defined paper point tips that absorbed a standard cytokine solution of a known concentration. These were stored in tubes filled with 350 μl PBS + 10% FCS. After sample assessment, a calibration curve was generated for recovery ELISA standards with the paper points. Additionally, a standard curve was generated for the volume adsorbed by the paper point tips up to the defined lines and the dilution factor in the buffer volume of 350 μl PBS + 10% FCS was calculated accordingly, to estimate the original concentration. All samples were thawed only once for analysis. Cytokines were detected by ELISA as per the manufacturer's instructions (BD Pharmingen, Heidelberg, Germany; R&D Systems, Minneapolis, USA). BD OptEIA antibody pairs were used to measure IL-1β, IL-6, and TNF-α (BD Pharmingen, Heidelberg, Germany). ELISA results were quantified using an Ultra384 ELISA reader (Tecan, Männedorf, Switzerland). The final cytokine concentrations were calculated by multiplication with the dilution factor (88.5) of the storage buffer and the defined recovery coefficient. Detection limits were defined in the same way by using the lowest positive standard of the respective ELISA.

Statistical analysis

Analyses were performed using GraphPad Prism 6 software for Mac (GraphPad Software Inc., La Jolla, CA, USA) running on Apple OS X. Variables were analyzed using two-way analysis of variance (ANOVA) for the comparison of immunological values. Time point and tooth/

Table 1. Study design for the experimental gingivitis/mucositis.

Week	0	4	4	5	6	7	8	9	10
Session	1 Oral prophylaxis, oral hygiene instructions	Perfect oral hygiene	2 Baseline measurements obtained; oral hygiene cessation started	3 Oral hygiene cessation week 1	4 Oral hygiene cessation week 2	5 Reinstitution of perfect oral hygiene, oral prophylaxis, oral hygiene instructions	6	7	8
			CF	CF	CF	CF	CF	CF	CF

CF, crevicular fluid sampling.

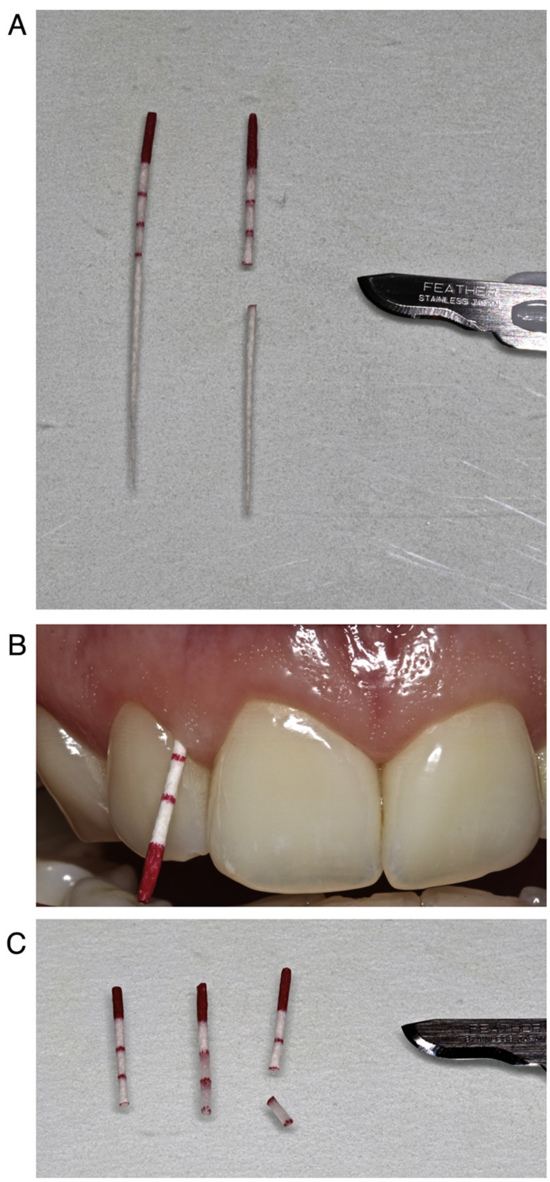

Fig. 1. (A) Sterile paper points with coloured bands (29 mm, ISO 25, taper .02; VDW) were used for gingival/peri-implant mucosal crevicular fluid sampling. Before assessment, the paper point was cut off at the lowest coloured band. (B) Clinical evaluation with a sterile paper point with coloured bands. (C) After sampling gingival/peri-implant mucosal crevicular fluid until it reached the second of the remaining coloured bands, the defined tip between two bands was cut off and stored.

implant type were included in the model, along with an interaction between the two factors, to determine whether the response differed over time for the three types of surface. Additionally, a post hoc Tukey multiple comparison test was used to identify any differences between the mean values of the groups. Any effect in the statistical model was classified as significant if the corresponding *P*-value fell below the 5% margin.

Results

A total of 16 patients (seven male and nine female) with 32 implants (16 zirconia and 16 titanium) were investigated (Table 2); their mean age at the time of study assessment was 57 years. On average, the implants had been in function for 2.1 years (minimum 1 year, maximum 5 years).

Immunological parameters

For the calibration of paper points, a curve for recovery ELISA standards was generated. Immunological parameters were measured from session 2 at week 4 to session 8 at week 10.

There was a significant difference in IL-6 between the groups (zirconia vs. tooth, $P = 0.045$; titanium vs. tooth, $P = 0.022$), with unfavourable values for the tooth unit (Fig. 2A). Additionally, IL-6 increased significantly around the teeth between session 2 at week 4 and session 4 at week 6 ($P = 0.012$), and this was followed by a decrease in value between session 4 at week 6 and session 6 at week 8 ($P = 0.030$). The lowest standard parameter for IL-6 (detection limit) was set at 1248 pg/ml.

TNF-α levels in the tooth samples from session 8 at week 10 were significantly higher than those in the zirconia implant samples ($P = 0.036$) and titanium implant samples ($P = 0.044$) (Fig. 2B). The lowest standard parameter for TNF-α (detection limit) was set at 2071 pg/ml.

There was a significant difference in IL-1β between the groups (zirconia vs. titanium, $P = 0.028$; titanium vs. tooth, $P < 0.05$), with titanium implant samples presenting higher IL-1β values (Fig. 2C). Furthermore, titanium implants presented a significant increase in IL-1β up to session 7 at week 9 ($P = 0.001$), with a subsequent decrease in session 8 at week 10 ($P = 0.009$). A statistically significant difference in IL-1β over time was evident ($P = 0.005$).

No statistically significant interactions between time and sampling units were observed for the immunological parameters.

Discussion

In 1965, Löe et al. presented a model of gingivitis around natural teeth and proved that the accumulation of bacterial plaque was the cause of gingival inflammation[9]. Within days of reinitiating oral hygiene methods, all patients were observed to have restored the healthy state of their gums, regardless of how long it took them to clinically develop gingivitis[9]. These findings also apply to the reactions of the mucosae to new plaque accumulation around implants[10].

At baseline, no differences were observed in the expression of any of the cytokines assessed around the implants and the teeth. This is in agreement with the findings of previous studies, which have shown that the expression of cytokines does not differ between implants and the teeth under ideal clinical conditions[11–14]. With the development of gingivitis/mucositis, significant but reversible changes were observed for several inflammation-associated biomarkers assessed around the implants and the teeth, as the values returned to baseline levels by the end of the experiment. The association between soft tissue inflammation and the expression of inflammation-associated biomarkers around the teeth and implants has been demonstrated previously[14,15]. Recent clinical data on zirconia implants have shown favourable peri-implant hard and soft tissue behaviour[16–21].

In a mucositis model that compared inflammation around titanium implants

Table 2. Implant and tooth positions. All implants were placed in the upper jaw.

	Position														Total
	17	16	15	14	13	12	11	21	22	23	24	25	26	27	
Zirconia implant			2	1		3	1	1	3	2	1	2			16
Titanium implant		1	1	3	2	1		1	1		1	2	2	1	16
Tooth		1		1	5	1	1	1	3	1	2				16

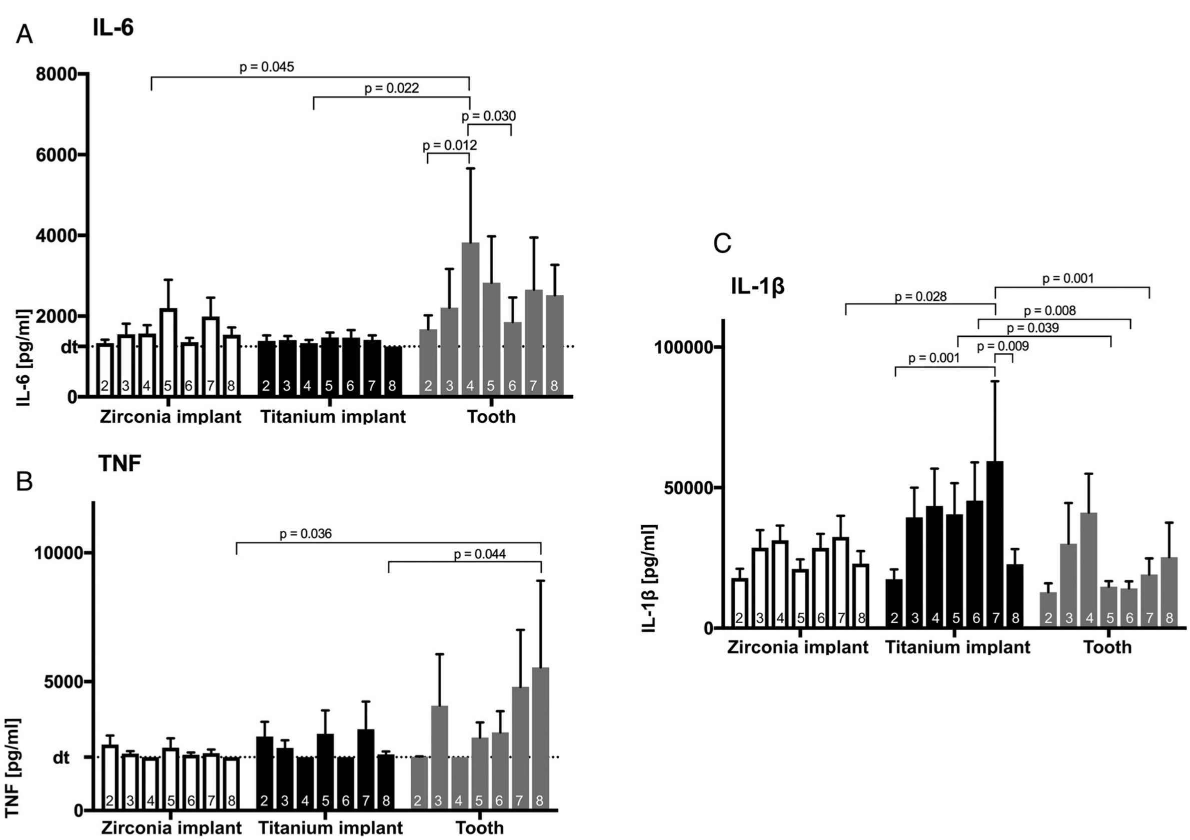

Fig. 2. (A) Mean IL-6 values measured around each zirconia implant, titanium implant, and natural tooth over time: sessions 2 (week 4), 3 (week 5), 4 (week 6), 5 (week 7), 6 (week 8), 7 (week 9), and 8 (week 10); the detection limit was 1248 pg/ml. (B) Mean TNF-α values measured around each zirconia implant, titanium implant, and natural tooth over time: sessions 2 (week 4), 3 (week 5), 4 (week 6), 5 (week 7), 6 (week 8), 7 (week 9), and 8 (week 10); the detection limit was 2071 pg/ml. (C) Mean IL-1β values measured around each zirconia implant, titanium implant, and natural tooth over time: sessions 2 (week 4), 3 (week 5), 4 (week 6), 5 (week 7), 6 (week 8), 7 (week 9), and 8 (week 10).

with experimental gingivitis around the teeth, Salvi et al. presented data showing that levels of gingival and peri−implant crevicular fluid markers, such as matrix metalloproteinase 8 (MMP−8) and IL−1β, increased in response to experimental plaque accumulation, compared with the levels for natural teeth[12]. Inflammation was clinically more pronounced in peri−implant soft tissue after the plaque accumulation phase. Experimental gingivitis and peri−implant mucositis were reversible in accordance with the host−derived parameters[12].

Regarding the levels of biomarkers, standardizing/equalizing the volume of crevicular fluid in the samples is essential, otherwise no valuable or comparable unit data can be obtained. Salvi et al. used paper strips to absorb crevicular fluid for 30 seconds[12]. However, they did not assess quantitative changes in fluid composition. The Periotron, an instrument designed to quantify sub−microlitre volumes of fluid sampled on a filter paper strip, was used in another study[22]. A disadvantage of this approach relates to the calibration, which was consistent for 1 week, but with incorrect use, the results were prone to error[22]. The present study used sterile paper points with standardized coloured bands. Before assessment, the paper points were cut off with a sterile scalpel at the lowest coloured band. The crevicular fluid was then sampled until it reached the second of the remaining coloured bands, following which the defined tip between two band marks was cut off. The level of fluid in the paper point and thus the end−point of sampling was clearly visible. Cutting of the paper points was performed on a sterile glass plate. A calibration curve was then generated. With the assumption that a defined length of paper always has the same capacity, the quantification of volumes of fluid samples was possible.

There was a significant difference in IL-6 between the groups (zirconia vs. tooth and titanium vs. tooth), with favourable values for both implant types. Evidence has shown that IL-6 polymorphisms are involved in soft tissue infections[23,24]. However, the implants performed better than the natural teeth. In a previous study on mucositis, significantly greater expression of IL-6 was seen around implant sites than around healthy teeth[25].

In addition, there were significant differences in IL-1β between the groups (zirconia vs. titanium and titanium vs. tooth), with lower IL-1β values for the zirconia implants and teeth. These results differ from those obtained in another study[12], which found no significant difference in the expression of IL-1β between implants and teeth with the development of inflammation. Other research on the development of gingivitis/mucositis has shown the opposite, with the most significant difference between implants and the teeth, and with higher levels of IL-1β obtained around the teeth[26,27]. Several studies using a gingivitis model have shown that IL-1β values around implants return to pre-experimental levels after performing proper hygiene[6,12,28]. Additionally, after the reinstitution of proper hygiene in the present study, IL-1β values (Fig. 2C) around the natural teeth immediately returned to pre-experimental values, although this was not statistically significant, whereas the increased concentrations remained around dental implants. This outcome is consistent with the clinical observation that peri-implant mucositis is less reversible than gingivitis. Furthermore, the study data are in line with the results presented by Salvi et al., which showed a more rapid return to initial IL-1β values around natural teeth than around implants after the reinstitution of proper hygiene[12]. The lower IL-1β values around zirconia implants might be due to the different implant surface, indicating less favourable results for the metallic titanium surface.

The findings of a recent systematic review revealed that IL-1β has been the cytokine most often studied, followed by TNF-α[29]. With regard to the release of TNF-α, significant differences were found between implants associated with healthy conditions and implants associated with peri-implantitis[30]. TNF-α is a proinflammatory cytokine that promotes bone resorption and mediates the inflammatory response to infection. In a study by Darabi et al., the significantly higher levels of TNF-α in patients with peri-implantitis than in patients in the control group indicated the key role of TNF-α in peri-implantitis[30]. Other studies have found that TNF-α polymorphism is not significantly associated with the risk of dental peri-implant disease[31,32]. However, further studies with large sample sizes should be performed to verify these results. Meyer et al. assessed the effect of a 3-week abstention from oral hygiene and found that IL-1β increased significantly with plaque accumulation[27]. IL-1β and TNF-α were significantly higher around teeth than around implants. At the 3-week follow-up, the levels of all biomarkers assessed had returned to baseline values. In the present study, TNF-α values were significantly lower around the implants than around the teeth.

The findings of this study showed consistent values around zirconia implants and the teeth, as well as increasing values around the titanium implants after the experimental plaque accumulation, for IL-1β and IL-6; the highest TNF-α values were measured around the teeth at the last session (session 8 at week 10). In another study evaluating zirconia and titanium implants, the levels of IL-1β, IL-6, and TNF-α did not differ significantly between the two implant types[13]. However, a critical consideration with regard to the present study is that the two different implants were not placed at the same appointment. Nevertheless, no implant presented any signs of infection prior to the investigation.

In conclusion, an association between experimental plaque accumulation and soft tissue inflammation was confirmed. The soft tissues around titanium implants developed a stronger inflammatory response to experimental plaque accumulation in terms of IL-1β, whereas the teeth presented an increase in IL-6 and TNF-α.

Acknowledgements. This work was performed at the Friedrich-Alexander-University Erlangen-Nürnberg (FAU) in fulfilment of the requirements for a Dr. med. dent. degree (Kim Clever). The authors thank Birgit Plümäkers (Institute of Immunology, University Hospital RWTH Aachen, Germany) for excellent technical assistance.

Funding. The authors declare no sources of funding.

Competing interests. The authors declare no conflicts of interest.

Ethical approval. Ethical approval was obtained (LMU University Munich 164–16).

Patient consent. Written patient consent was obtained.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijom.2018.10.018

References

1. Schneider C, Zemp E, Zitzmann NU. Oral health improvements in Switzerland over 20 years. *Eur J Oral Sci* 2017;**125**:55–62.
2. Albrektsson T, Dahlin C, Jemt T, Sennerby L, Turri A, Wennerberg A. Is marginal bone loss around oral implants the result of a provoked foreign body reaction. *Clin Implant Dent Relat Res* 2014;**16**:155–65.
3. Gavira JA. Current trends in protein crystallization. *Arch Biochem Biophys* 2016;**602**:3–11.
4. Christel P, Meunier A, Heller M, Torre JP, Peille CN. Mechanical properties and short-term in-vivo evaluation of yttrium-oxide-partially-stabilized zirconia. *J Biomed Mater Res* 1989;**23**:45–61.
5. Khammissa RA, Feller L, Meyerov R, Lemmer J. Peri-implant mucositis and peri-implantitis: clinical and histopathological characteristics and treatment. *SADJ 2012* 2016;**67**(122):124–6.
6. Heitz-Mayfield LJA, Salvi GE. Peri-implant mucositis. *J Periodontol* 2018;**89**(Suppl 1): S257–66.
7. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, Poole C, Schlesselman JJ, Egger M. Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration. *Epidemiology* 2007;**18**:805–35.
8. Bass CC. An effective method of personal oral hygiene. *J La State Med Soc* 1954;**106**:57–73. contd.
9. Löe H, Theilade E, Jensen SB. Experimental gingivitis in man. *J Periodontol* 1965;**36**:177–87.
10. Zitzmann NU, Berglundh T, Marinello CP, Lindhe J. Experimental peri-implant mucositis in man. *J Clin Periodontol* 2001;**28**:517–23.
11. Tsalikis L. The effect of age on the gingival crevicular fluid composition during experimental gingivitis. A pilot study. *Open Dent J* 2010;**4**:13–26.
12. Salvi GE, Aglietta M, Eick S, Sculean A, Lang NP, Ramseier CA. Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans. *Clin Oral Implants Res* 2012;**23**:182–90.
13. Cionca N, Hashim D, Cancela J, Giannopoulou C, Mombelli A. Pro-inflammatory cytokines at zirconia implants and teeth: a cross-sectional assessment. *Clin Oral Investig* 2016;**20**:2285–91.
14. Ramseier CA, Eick S, Bronnimann C, Buser D, Bragger U, Salvi GE. Host-derived biomarkers at teeth and implants in partially edentulous patients. A 10-year retrospective study. *Clin Oral Implants Res* 2016;**27**:211–7.
15. Guncu GN, Akman AC, Gunday S, Yamalik N, Berker E. Effect of inflammation on cytokine levels and bone remodelling markers in peri-implant sulcus fluid: a preliminary report. *Cytokine* 2012;**59**:313–6.
16. Kniha K, Schlegel KA, Kniha H, Modabber A, Holzle F, Kniha K. Evaluation of peri-implant bone levels and soft tissue dimensions around zirconia implants—a three-year follow-up study. *Int J Oral Maxillofac Surg* 2018;**47**:492–8.
17. Kniha K, Modabber A, Kniha H, Mohlhenrich SC, Holzle F, Milz S. Dimensions of hard and soft tissue around adjacent, compared with single-tooth, zirconia implants. *Br J Oral Maxillofac Surg* 2018;**56**:43–7.
18. Kniha K, Milz S, Kniha H, Ayoub N, Holzle F, Modabber A. Peri-implant crestal bone changes around zirconia implants in periodontally healthy and compromised patients. *Int J Oral Maxillofac Implants* 2018;**33**: 217–22.
19. Kniha K, Kniha H, Mohlhenrich SC, Milz S, Holzle F, Modabber A. Papilla and alveolar crest levels in immediate versus delayed single-tooth zirconia implants. *Int J Oral Maxillofac Surg* 2017;**46**:1039–44.
20. Kniha K, Gahlert M, Hicklin S, Bragger U, Kniha H, Milz S. Evaluation of hard and soft tissue dimensions around zirconium oxide implant-supported crowns: a 1-year retrospective study. *J Periodontol* 2016;**87**: 511–8.
21. Kniha K, Schlegel KA, Kniha H, Modabber A, Neukam F, Kniha K. Papilla–crown height dimensions around zirconium dioxide implants in the esthetic area: a 3-year follow-up study. *J Prosthodont* 2018;**28**(Jan). [Epub ahead of print].
22. Ciantar M. Caruana DJ. Periotron 8000: calibration characteristics and reliability. *J Periodontal Res* 1998;**33**:259–64.
23. Severino VO, Napimoga MH, de Lima Pereira SA. Expression of IL-6, IL-10, IL-17 and IL-8 in the peri-implant crevicular fluid of patients with peri-implantitis. *Arch Oral Biol* 2011;**56**:823–8.
24. Scapoli L, Girardi A, Palmieri A, Martinelli M, Cura F, Lauritano D, Pezzetti F, Carinci F. Interleukin-6 gene polymorphism modulates the risk of periodontal diseases. *J Biol Regul Homeost Agents* 2015;**29**:111–6.
25. Ata-Ali J, Flichy-Fernandez AJ, Ata-Ali F, Penarrocha-Diago M, Penarrocha-Diago M. Clinical, microbiologic, and host response characteristics in patients with peri-implant mucositis. *Int J Oral Maxillofac Implants* 2013;**28**:883–90.
26. Hall J, Pehrson NG, Ekestubbe A, Jemt T, Friberg B. A controlled, cross-sectional exploratory study on markers for the plasminogen system and inflammation in crevicular fluid samples from healthy, mucositis and peri-implantitis sites. *Eur J Oral Implantol* 2015;**8**:153–66.
27. Meyer S, Giannopoulou C, Courvoisier D, Schimmel M, Muller F, Mombelli A. Experimental mucositis and experimental gingivitis in persons aged 70 or over. Clinical and biological responses. *Clin Oral Implants Res* 2017;**28**:1005–12.
28. Schierano G, Pejrone G, Brusco P, Trombetta A, Martinasso G, Preti G, Canuto RA. TNF-alpha TGF-beta2 and IL-1beta levels in gingival and peri-implant crevicular fluid before and after de novo plaque accumulation. *J Clin Periodontol* 2008;**35**:532–8.
29. Faot F, Nascimento GG, Bielemann AM, Campao TD, Leite FR, Quirynen M. Can peri-implant crevicular fluid assist in the diagnosis of peri-implantitis? A systematic review and meta-analysis. *J Periodontol* 2015;**86**:631–45.
30. Darabi E, Kadkhoda Z, Amirzargar A. Comparison of the levels of tumor necrosis factor-alpha and interleukin-17 in gingival crevicular fluid of patients with peri-implantitis and a control group with healthy implants. *Iran J Allergy Asthma Immunol* 2013;**12**:75–80.
31. Mo YY, Zeng XT, Weng H, Cen Y, Zhao Q, Wen X. Association between tumor necrosis factor-alpha G-308A polymorphism and dental peri-implant disease risk: a meta-analysis. *Med (Baltimore)* 2016;**95**:e4425.
32. Cury PR, Joly JC, Freitas N, Sendyk WR, Nunes FD, de Araujo NS. Effect of tumor necrosis factor-alpha gene polymorphism on peri-implant bone loss following prosthetic reconstruction. *Implant Dent* 2007;**16**:80–8.

Address:
Department of Oral and Cranio-Maxillofacial Surgery
University Hospital RWTH Aachen
Pauwelsstraße 30
52074 Aachen
Germany
Tel: +49 2418088258
E-mail: kkniha@ukaachen.de