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Early bacterial colonization and soft tissue health around zirconia and titanium abutments: an *in vivo* study in man

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Key words: abutment, implant, microbiology, peri-implant mucosa, titanium, zirconia

Abstract

Aim: To compare the early bacterial colonization and soft tissue health of mucosa adjacent to zirconia (ZrO_2) and titanium (Ti) abutment surfaces *in vivo*.

Materials and methods: Twenty edentulous subjects received two endosseous mandibular implants. The implants were fitted with either a ZrO_2 or a Ti abutment (non-submerged implant placement, within-subject comparison, left-right randomization). Sulcular bacterial sampling and the assessment of probing pocket depth, recession and bleeding on probing were performed at 2 weeks and 3 months post-surgery. Wilcoxon matched-pairs, sign-rank tests were applied to test differences in the counts of seven marker bacteria and the clinical parameters that were associated with the ZrO_2 and Ti abutments, at the two observation time points.

Results: ZrO_2 and Ti abutments harboured similar counts of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Peptostreptococcus micros*, *Fusobacterium nucleatum* and *Treponema denticola* at 2 weeks and 3 months. Healthy clinical conditions were seen around both ZrO_2 and Ti abutments at all times, without significant differences in most clinical parameters of peri-implant soft tissue health. Mean probing depths around Ti abutments were slightly deeper than around ZrO_2 abutments after 3 months (2.2 SD 0.8 mm vs. 1.7 SD 0.7 mm, $P = 0.03$).

Conclusions: No difference in health of the soft tissues adjacent to ZrO_2 and Ti abutment surfaces or in early bacterial colonization could be demonstrated, although somewhat shallower probing depths were observed around ZrO_2 abutments after 3 month.

Introduction

Titanium (Ti) has been the ''gold standard'' material for implant abutments, but the use of high-strength ceramics, both as permucosal abutments on implants and as copings for ceramic crowns, is increasing. Zirconia (ZrO_2) is especially promising because of its high fracture toughness and favourable light dynamics. To date, there is only limited information available with respect to the clinical and biological performance of ZrO_2-based restorations (Jung et al. 2008; Sailer et al. 2009b; Zembic et al. 2009a). Attention in the literature has predominantly been focused on the bone–implant response to Ti and ZrO_2 and on the biomechanical properties of these materials (Wenz et al. 2008). Much less information is available regarding the soft tissue response to ZrO_2 and comparative *in vivo* studies in humans are quite scarce (Myshin & Wiens 2005; Teughels et al. 2006b; Linkevicius & Apse 2008a).

The establishment and maintenance of healthy soft tissues around implant abutments are considered to be important for the long-term service of the implant (Berglundh et al. 1991; Lindquist et al. 1996). The intimate contact between the marginal mucosa and implant abutment protects the implant body from the microbial communities of the mouth. As on teeth, periodontal pathogens on implants induce soft tissue infection (Zitzmann et al. 2002). It is presumed that this may jeopardize the osseointegration process (Norowski & Bumgardner 2009a).

The adhesion, proliferation and colonization of cells and micro-organisms are dependent upon the surface properties, among which are its biocompatibility (i.e. chemistry), surface topography (i.e. roughness) and surface-free energy (Quirynen et al. 1993, 1994; Bollen et al. 1996a; Rimondini et al. 1997; Abrahamsson et al. 1998, 2002; Rasperini et al. 1998; Grossner-Schreiber et al. 2001a; Hamdan et al. 2006; Rompen et al. 2006; Teughels et al. 2006a; Linkevicius & Apse 2008b). Bacterial colonization of the abutment starts directly after exposure to the oral environment and within weeks, the

subgingival microbiota is similar to that found around teeth in the same mouth (van Winkelhoff et al. 2000; Quirynen et al. 2005, 2006b; DeAngelo et al. 2007; Furst et al. 2007a; Salvi et al. 2008).

Strategies aimed at reducing bacterial adhesion and biofilm formation on implant abutment surfaces are of pertinent clinical interest and can be used for the maintenance of soft tissue health or possibly in the treatment of peri-implantitis. Recent studies have shown that antimicrobial (e.g. vancomycin or chitosan) derivatization of a Ti alloy surface renders it less susceptible for bacterial colonization *in vitro* (Parvizi et al. 2004; Antoci et al. 2008; Shi et al. 2008). Implant coatings that deliver antibiotics have been described as well, predominantly in the field of orthopaedics (Norowski & Bumgardner 2009b). It was shown that the physical properties of the Ti surface can be adapted, for example by applying a coating of Ti-nitride through vapour deposition. This reduces plaque adhesion compared with uncoated Ti surfaces both *in vitro* and *in vivo* (Grossner-Schreiber et al. 2001b; Scarano et al. 2003) and still facilitates cellular adhesion of human fibroblasts *in vitro* (Grossner-Schreiber et al. 2006). In addition, it has been observed that silver and zinc oxide-modified surfaces possess antibacterial properties as well (Norowski & Bumgardner 2009c).

Wennerberg and colleagues compared the inflammatory response in human peri-implant mucosa around standard Ti abutments and abutments that were roughened by grid blasting. They found no correlation between the number of inflammatory cells and the degree of roughness after 4 weeks (Wennerberg et al. 2003). However, creating much smoother surfaces than those generally encountered on currently used Ti abutments (R_a–value approximately 35 nm) reduces bacterial adhesion *in vitro* (Pier-Francesco et al. 2006). Other authors compared Ti abutments with different roughnesses and a smooth ceramic abutment (of undisclosed chemical composition, R_a-value 60 nm). Because fibroblasts require a certain roughness to be able to adhere to a Ti substrate, the authors suggest an optimal surface roughness R_a-value of 200 nm. Such roughness constitutes a good balance. It reduces plaque adhesion as compared with a rougher surface, yet is still rough enough for fibroblast adhesion and the establishment of a durable epithelial soft tissue seal (Bollen et al. 1996b; Quirynen et al. 1996). Interestingly, no difference in early biofilm formation on subgingival abutment surfaces with varying roughnesses could be demonstrated by others (Elter et al. 2008b).

The potential advantages of ZrO_2 compared with Ti, with respect to biofilm formation in the oral cavity, has been demonstrated in various studies. ZrO_2 discs that were glued on a device and worn intra-orally for a day elicited less plaque accumulation than Ti discs *in vivo* (Scarano et al. 2004a). This finding was attributed to the superficial structure of the ZrO_2, more specifically, to its electric conductivity. Others reported similar favourable findings *in vitro* and *in vivo* in a comparable experiment (Rimondini et al. 2002a). These observations were not verified on functional, permucosal abutments. Degidi and colleagues performed a study in five patients comparing ZrO_2 and Ti in permucosal applications. Less pronounced inflammation-related processes were noticed around ZrO_2 vs. Ti healing abutments after 6 months (Degidi et al. 2006). The peri-implant microbiota was not investigated in the latter study.

The present investigation focuses on the peri-implant mucosa condition adjacent to ZrO_2 and Ti abutment surfaces and on early submucosal bacterial colonization. These issues are compared under the null hypotheses that permucosal sites adjacent to ZrO_2 and Ti abutment surfaces exhibit similar clinical characteristics of peri-implant soft tissue health and microbiological features during the first 3 months.

Materials and methods

The study was designed as a prospective, human, within-subject comparison with left–right randomization. Twenty edentulous patients, nine males and 11 females, aged between 39 and 76 years (mean 56.4 years) who were scheduled for two mandibular implants and overdenture treatment, were enrolled in the study. Inclusion criteria were:

- reasonable-to-good general health, as expressed by a score I or II on the physical status classification system by the American Association of Anesthesiologists (ASA-score);
- bone height in the mandibular anterior region allowing the placement of 11, 13 or 15 mm screw implants. Bone width had to be such that implants of 3.5 or 4 mm in diameter could be placed;
- no history of previous implant loss, no pathology or irradiation of the (anterior) mandible.

The study protocol was approved by the medical ethics committee of the University Medical Center Utrecht and written informed consent was obtained.

Implant installation

Two Ti screw implants (OsseoSpeed™ Implants, Astra Tech AB, Mölndal, Sweden) were placed in local anaesthesia in the region of the former mandibular cuspids. Subjects received antibiotics (Vibramycin, from 1 day pre-operatively 200 mg until 7 days post-operatively, once daily 100 mg) and rinsed with a 0.2% chlorhexidine solution from 2 days pre-operatively until 2 weeks post-operatively.

Implant diameter and length within each subject were similar. The implants were placed and randomized to immediately be provided with either one (experimental) ZrO_2 or one Ti abutment, functioning as a permucosal healing abutment.

Two weeks after surgery, brushing was allowed. Subjects were enrolled in a strict follow-up protocol that focused on oral hygiene, but during the experimental period, the abutments were never professionally cleaned.

Abutments (ZrO_2 and Ti)

The experimental abutments were especially designed, fabricated and CE-marked for the study and are not commercially available. Bulk material for the Ti abutments was Ti, grade 4, according to ASTM F-67 and Y-TZP according to ISO 13356 for the ZrO_2 specimen (Astra Tech AB). Abutment materials and production methods were basically similar to those used in the production of commercially available, regular Ti and ZrO_2 abutments by the same manufacturer (i.e. the ZirDesign™ and TiDesign™ abutments, Astra Tech AB). Surface finish requirements for both abutment types were also similar to ordinary production.

The surface roughness of the experimental ZrO_2 and Ti abutments was measured at three locations on one specimen of each material by means of contact profilometry. Mean R_a-values were 236 nm (range: 217–255 nm) for the ZrO_2 abutment and 210 nm (range: 173–272 nm) for the Ti abutment. The corresponding R_q-values were 292 nm (range: 260–330 nm) and 259 nm (range: 220–332 nm) for the ZrO_2 and Ti abutments. Hence, the surface roughness of the materials used was considered to be in the same order of magnitude, and the main difference between the two experimental abutments is their chemical composition.

The location for the ZrO_2 and Ti abutment (left/right) was allotted at random in such a way that the distribution over the 20 patients resulted in a balanced design.

Microbiological sampling and follow-up

Microbiological sampling and measurement of clinical parameters were performed at 2 weeks and 3 months post-operatively. Sulcular plaque samples were obtained by performing a circumferential motion (360°) in the peri-implant sulci

with a sterilized single-use plastic scaler (Implacare®, Hu-Friedy, Rockwell st, Chicago, IL, USA).

Microbiological analysis

Detection and counting of the numbers of *Aggregatibacter actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis* (*Pg*), *Prevotella intermedia* (*Pi*), *Tannerella forsythia* (*Tf*), *Parvimonas micra* (*Pm*), *Fusobacterium nucleatum* (*Fn*) and *Treponema denticola* (*Td*) were performed using real-time PCR as described by others (Kuboniwa et al. 2004a; Boutaga et al. 2005b). In brief, amplification of species-specific 16S rDNA sequences was performed in a 20 μl reaction mixture containing 10 μl of 2 × LightCycler® 480 Probes Master (Roche, Indianapolis, IN, USA), 300 nM of species-specific primers, 100 nM of a species-specific probe (both from TIB MolBiol GmbH, Berlin, Germany; modified by a FAM reporter and a BHQ-2 quencher) and 5 μl of DNA purified from the plaque samples. The sequences of species-specific primers and probes have been described by Boutaga et al. (2003, 2005a) and those for *T. denticola* by Kuboniwa et al. (2004b). Five microlitres of the DNA extracted from the following well-defined reference strains was used to prepare a standard curve as positive controls: *P. gingivalis* strain HG66 (W83), *T. forsythia* ATCC 43037, *A. actinomycetemcomitans* NCTC 9710, *P. intermedia* ATCC 25611, *F. nucleatum* ATCC 25586, *P. micra* HG 1179 (ATCC 33270) and *T. denticola* (ATCC 33520); 5 μl of sterile H_2O was used as a non-template control.

The samples were subjected to an initial single incubation at 95°C for 10 min, followed by 45 cycles at 95°C for 10 s and 60°C for 20 s. DNA amplification was monitored by quantitatively analysing the fluorescence emission (LightCycler 480, software version 1.5, Roche) during each annealing–extension step.

Clinical parameters

Probing pocket depth (PPD), recession (REC) and bleeding on probing (BOP) were assessed at two sites per implant (mid-buccal and mesial). A plastic periodontal probe with 0.25 N of calibrated probing force was used (Click-probe®, KerrHawe, Bioggio, Switzerland). PPD was measured in millimeters from the mucosal margin to the clinical pocket. REC was measured in millimeters from the edge of the abutment to the mucosal margin (Fig. 1). BOP was recorded as absent (score = 0) or present (score = 1). Mean values per implant were calculated for the continuous parameters (meanPPD, meanREC). BOP is presented as the percentage of implants that demonstrated either mid-buccal or mesial BOP.

Statistical analysis

The mean values for the clinical parameters and levels of the seven marker bacteria associated with the ZrO_2 and Ti abutments were described and statistically compared at 2 weeks and after 3 months post-surgery. Non-parametric statistical procedures were used for all comparisons (Wilcoxon matched-pairs, sign-rank test). All statistical computations were performed in a standard statistical program (SPSS version 16, SPSS Inc., Chicago, IL, USA). Statistical significance of the comparison between the ZrO_2 and Ti abutments and the two observation periods was set at $P<0.05$.

Results

Data at 3 months in one subject could not be recorded because of a breach of protocol. The experimental abutments had already been removed before microbiological sampling and clinical measurement taking.

Mean values for the clinical parameters of the peri-implant mucosa surrounding the ZrO_2 and Ti abutments at 2 weeks and at 3 months are presented in Table 1 (meanPPD, meanREC and BOP). Mean probing depths at 3 months were shallower around ZrO_2 compared with Ti abutments. No further statistically significant clinical differences between ZrO_2 and Ti abutments were observed for meanPPD, meanREC or BOP at 2 weeks or 3 months. The meanPPD decreased significantly for both the ZrO_2 and the Ti abutments between 2 weeks and 3 months. In contrast, meanREC increased in time for both abutment types. Slightly less BOP was observed around the Ti abutments at 3 months compared with the observations 2 weeks post-operatively (Table 1).

The numbers of peri-implant sites with detectable levels of seven periodontal bacteria at 2 weeks and at 3 months are presented in Table 2. The cumulative bacterial load is described per subject in Table 3. No statistically significant difference could be observed in counts of the 7 marker bacteria or in cumulative bacterial load between the ZrO_2 and Ti abutments, both at 2 weeks and at 3 months. Generally, slightly larger numbers of bacteria were found at the ZrO_2 abutment surfaces compared with the Ti surfaces, although this never reached a statistically significant level (Table 2).

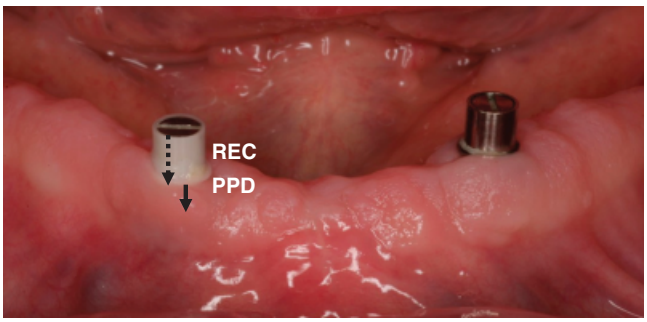

Fig. 1. Zirconia and titanium experimental abutments *in situ* after 3 months. Recession (REC) is measured from the edge of the implant to the mucosal margin. The pocket probing depth (PPD) is measured from the mucosal margin to the clinical pocket.

Table 1. **Evaluation of mean pocket probing depth (meanPPD), mean recession (meanREC) and bleeding on probing (BOP, either buccal or mesial)**

	2 weeks	3 months	*P*-level
MeanPPD			
ZrO_2	3 (1.1)	1.7 (0.7)	$Z_{-3.65}$, $P=0$
Ti	2.9 (0.8)	2.2 (0.8)	$Z_{-3.01}$, $P=0$
	$Z_{0.14}$, $P=0.89$	$Z_{-2.16}$, $P=0.03$	
MeanREC			
ZrO_2	2.1 (1.2)	2.7 (0.6)	$Z_{-2.49}$, $P=0.01$
Ti	1.9 (1.2)	2.6 (1)	$Z_{-2.82}$, $P=0$
	$Z_{-0.97}$, $P=0.14$	$Z_{-0.32}$, $P=0.98$	
BOP			
ZrO_2	50%	52.6%	$Z_{-0.25}$, $P=0.8$
Ti	75%	47.4%	$Z_{-2.01}$, $P=0.05$
	$Z_{-0.83}$, $P=0.41$	$Z_{-1.19}$, $P=0.23$	

Pairwise comparison of data after 2 weeks and 3 months for zirconia (ZrO_2) and for titanium (Ti) abutments (Wilcoxon matched-pairs test, sign-rank test). Standard deviations between brackets ($n=20$ subjects for the 2 weeks and 19 subjects for the 3-month interval).

Table 2. **The number of peri-implant sites with detectable levels of seven periodontal bacterial species using RT PCR, 2 weeks and 3 months after installation of the zirconia (ZrO_2) and titanium (Ti) abutments ($n = 20$ subjects for the 2 weeks and $n = 19$ subjects for the 3 month interval)**

	Aa		*Pg*		*Pi*		*Tf*		*Pm*		*Fn*		*Td*	
	ZrO_2	Ti	ZrO_2	Ti	ZrO_2	Ti	ZrO_2	Ti	ZrO_2	Ti	ZrO_2	Ti	ZrO_2	Ti
2 weeks ($n = 20$)														
N detected	1	2	0	0	3	1	1	0	7	6	17	15	2	1
ZrO_2+/Ti−	1		0		3		1		4		2		2	
ZrO_2-/Ti+	2		0		1		0		3		1		1	
ZrO_2 = Ti	17		20		16		19		13		17		17	
Mean	4620	220	0	0	4150	91	22,000,000	0	45,395	16,184	126,465	13,467,002	49,503	280
SD	0	199	0	0	6802	0	0	0	94,798	33,530	434,701	4,810,665	69,999	0
Median	4620	220	0	0	440	91	22,000,000	0	4100	400	1540	1070	49,504	280
3 months ($n = 19$)														
N detected	0	0	1	2	4	5	1	0	11	11	17	17	2	0
ZrO_2+/Ti−	0		0		0		0		2		0		2	
ZrO_2-/Ti+	0		1		1		0		2		0		0	
ZrO_2 = Ti	19		18		18		19		15		19		17	
Mean	0	0	1,000,000	64,000	600,088	3,600,089*	3700	0	221,651	170,351*	1,728,753	702,662	280	0
SD	0	0	0	36,770	952,117	804,935	0	0	338,980	509,342	4,555,954	1,548,894	170	0
Median	0	0	1,000,000	64,000	200,090	42	3700	0	3800	2000	120,000	37,000	280	0

Mean absolute counts (mean) for those observations exceeding the detection threshold and their standard deviation (SD) as well as the median values are presented.
*Statistically significant difference between 2 weeks and 3 months, $P < 0.05$.

Table 3. **Cumulative bacterial load of seven periodontal bacterial species using RT PCR on zirconia and titanium abutment surfaces at 2 weeks and 3 months post surgery ($n = 20$ subjects for the 2 weeks and 19 subjects for the 3 month interval)**

Subject	2 weeks		3 months		ZrO_2		Ti	
	ZrO_2	Ti	ZrO_2	Ti	2 weeks	3 months	2 weeks	3 months
1	0	0	–	–	0	–	0	–
2	1700	69,000	1,810,000	106,000	1700	1,810,000	69,000	106,000
3	298,631	19,390,200	853,000	1,433,000	298,631	853,000	19,390,200	1,433,000
4	1540	1249	1,280,000	2830	1540	1,280,000	1249	2830
5	114	4260	400,019	2,260,000	114	400,019	4260	2,260,000
6	3006	266	690,620	2,000,862	3006	690,620	266	2,000,862
7	220,000	220,000	2300	2500	220,000	2300	220,000	2500
8	50,240	0	0	0	50,240	0	0	0
9	30,410	170	3300	220	30,410	3300	170	220
10	1,804,110	1420	124,200	112,200	1,804,110	124,200	1420	112,200
11	22,213,000	66,000	4900	2175	22,213,000	4900	66,000	2175
12	3230	290	3400	3400	3230	3400	290	3400
13	3100	1540	650,170	3830	3100	650,170	1540	3830
14	650	650	4,800,160	9,500,000	650	4,800,160	650	9,500,000
15	340	400	16,000	1200	340	16,000	400	1200
16	0	374	4,530,000	110,540	0	4,530,000	374	110,540
17	656	970	48,000	32,400	656	48,000	970	32,400
18	43,000	4500	3200	1500	43,000	3200	4500	1500
19	58	0	8600	38,900	58	8600	0	38,900
20	750	1,900,041	2,903,700	136,000	750	2,903,700	1,900,041	136,000
Summary	Ti > ZrO_2: 7		Ti > ZrO_2: 6		ZrO_2 (2 weeks) > ZrO_2 (3 months): 6		Ti (2 weeks) > Ti (3 months): 5	
statistic	Ti < ZrO_2: 10		Ti < ZrO_2: 11		ZrO_2 (2 weeks) < ZrO_2 (3 months): 13		Ti (2 weeks) < Ti (3 months): 13	
	Ti = ZrO_2: 3		Ti = ZrO_2: 2		ZrO_2 (2 weeks) = ZrO_2 (3 months): 0		Ti (2 weeks) = Ti (3 months): 1	
	$Z_{-0.59}$, $P = 0.55$		$Z_{-0.73}$, $P = 0.46$		$Z_{-1.53}$, $P = 0.13$		$Z_{-1.11}$, $P = 0.27$	

Data are presented per subject, in absolute counts per sample and pairwise compared (Wilcoxon matched-pairs, sign-rank test). Sites where the detection threshold was not exceeded are awarded the value "0".

At 2 weeks, the most frequently detected periodontal species were *P. micra* and *F. nucleatum*. In contrast, periodontal pathogens such *as A. actinomycetemcomitans, P. gingivalis* and *T. denticola* were not detectable in the majority of patients (Table 2). *A. actinomycetemcomitans* was detected in three subjects at 2 weeks post-surgery, but was no longer detectable at 3 months in any of test sites. Two subjects hosted *P. gingivalis* at 3 months, but not at 2 weeks.

The bacterial colonization of ZrO_2 surfaces did not undergo major changes between 2 weeks and 3 months, although a slight increase of *F. nucleatum* cells was observed ($Z_{-1.97}$, $P = 0.05$). At Ti abutment surfaces, the counts of *P. intermedia* ($Z_{-2.02}$, $P < 0.05$) and *P. micra* ($Z_{-2.10}$, $P < 0.05$) increased statistically significantly in time (Table 2).

Discussion

ZrO_2 is becoming a favoured material in restorative dentistry for implant abutments and as copings for crowns and bridges, mainly because of its presumed favourable light dynamics. In a way, this is somewhat worrying considering the fact that long-term clinical data documenting the performance of ZrO_2 abutments and restorations are scarce. The same can be said with respect to the soft tissue response to ZrO_2 itself, because well-controlled *in vivo* human studies are lacking as was also postulated in a consensus statement on soft tissue integration (Klinge & Meyle 2006). The present study deals with the peri-implant soft tissue response to ZrO_2 and Ti implant abutments and the early bacterial colonization.

The choice for a within-subject comparison in edentulous subjects was made because it offered the best possibility for eliminating confounding factors. For example, the bacterial challenge by the oral microflora is the same in one individual. As a result, implant dimensions and many other variables within the same subject were similar in all cases and microbiological sampling and clinical procedures could be standardized as much as possible. Because the surface roughness of the experimental abutments made from ZrO_2 and Ti was also more or less similar, potential differences in soft tissue response and in bacterial colonization are presumably the result of differences in the chemical composition and consequently of differences in surface-free energy (electrical conductivity). The surface roughness

of the abutments that were used (R_a-values 210–236 nm) approached the optimal roughness that was suggested in the literature for permucosal implant abutments (Bollen et al. 1996c; Quirynen et al. 2006a).

Only a few reports describe longitudinal changes of the subgingival microflora after changing substrata or during implantation (Lee et al. 1999; Furst et al. 2007b). In the present study, a detection method was chosen comprising high specificity and sensitivity towards pathogenic bacterial species that are related to peri-implant infection. Therefore, we have not performed an investigation method that gives an overview of ''all species'', like anaerobic culture or – a money-wise expensive – molecular technique as next-generation sequencing, although the latter would be a very promising option (Zaura et al. 2009). Another advantage of the chosen method was that it enabled us to really quantify the numbers of bacterial cells per species during the evaluation period. Other techniques as DNA–DNA checkerboard hybridization are semi-quantitative only. A real-time PCR is more precise in this way. However, it should be mentioned that a closed target method as real-time PCR might result in an underestimation of changes in bacterial colonization on both ZrO_2 and Ti surfaces.

In general, comparable microorganisms are found around newly placed implants and the remaining dentition. This can also include periodontopathogens as *P. gingivalis* and *A. actinomycetemcomitans*, which might even be a risk for future peri-implant infections (Leonhardt et al. 1999). However, it was to be expected that colonization of implants by such pathogens is restricted to partially edentulous patients, because of the remaining presence of a specific niche, e.g. the periodontal sulcus (van Winkelhoff et al., 2000). However, recent findings by Van Assche et al. (2009) using real-time PCR techniques to determine the presence of periodontopathogens reveal that such bacteria will remain at mucosal sites after full-mouth extraction. This might explain our observation of the presence of *A. actinomycetemcomitans* at implant sites in three edentulous patients and *P. gingivalis* in two patients.

As in the majority of clinical studies dealing with the evaluation of dental implants and soft tissue health, the condition of the peri-implant mucosa was monitored by means of PPD, REC and the assessment of a bleeding index (Lang et al. 2004). The use of such ''periodontal'' parameters to determine the clinical condition of the soft peri-implant tissues has been subject to debate (Ow et al. 1999; Verhoeven et al. 2000). These parameters were used because of the lack of reliable, more sensitive, clinical measures to assess the biological response of peri-implant mucosa. The effect of the antibiotics used perioperatively will presumably have affected the soft tissue response at the 2 weeks measurements and not so much so after 3 months. This will be the case for both abutments in a similar manner because of the split mouth study design.

No significant difference in the mean values for PPD were observed between ZrO_2 and Ti abutments at 2 weeks. However, at 3 months the permucosal seal around the ZrO_2 abutments appeared somewhat less sensitive to probe penetration as compared with that around the Ti abutments ($P = 0.03$). Because the two time points were analysed separately, the chance on false positive findings has increased and statistically significant observations with P-values in the vicinity of 0.05 should be interpreted with caution. In addition, it should be noted that the geometry of the abutments used (Fig. 1) may have played a role and hampered reliable probe penetration. A comparison with probing depth measurements, as obtained in other studies does not seem appropriate. The mean values for REC and BOP were more or less similar at all times for both materials. With respect to the latter, it is interesting to note that in a clinical study on the performance of ZrO_2 and Ti abutments after 1 and 3 years of function, slightly more BOP occurred around the ZrO_2 abutments as compared with the Ti abutments (Sailer et al. 2009a; Zembic et al. 2009b). This was not apparent in the present investigation. It has been suggested that the use of 0.25 N of calibrated probing force (Click-probe®, KerrHawe, Bioggio, Switzerland) induces epithelial bleeding in the absence of soft tissue infection (false positive observations) (Gerber et al. 2009).

Between 2 weeks and 3 months after implant installation, the permucosal tissues undergo some changes. Probing depths decrease and the amount of REC increases irrespective of the abutment type.

There was no significant difference between the ZrO_2 and Ti abutments either in the prevalence or in the counts of any of the seven marker bacteria, both at 2 weeks and at 3 months (Tables 2 and 3). Hence, a marked qualitative or quantitative difference in the early bacterial colonization of ZrO_2 and Ti abutment surfaces was not observed. In *in vitro* and *in vivo* studies where the colonization of bacteria was investigated on intra-orally worn ZrO_2 and Ti discs, which were embedded in removable prosthetic appliances, ZrO_2 discs harboured less bacteria (Rimondini et al. 2002b; Scarano et al. 2004b). Such a difference was not found in the present study which may be explained by the different techniques of sampling. It has been suggested that the use of intra-oral discs is confounded by tongue and cheek activity (Heuer et al. 2007a; Elter et al. 2008a).

In a study on early biofilm formation on implant abutments, *A. actinomycetemcomitans* and *P. gingivalis* were not detected on any of the 14 Ti healing abutments in 10 patients after 12 days. The authors did not disclose whether the subjects were partly or fully edentulous (Heuer et al. 2007b). In the present study *A. actinomycetemcomitans* and *P. gingivalis* were infrequently detected in a very small number of patients.

Overall, on the basis of the studied biological and microbial parameters there are no compelling grounds to favour one abutment material over the other after 3 months. Considering the limitations that are associated with the use of the rather robust parameters of soft tissue health that were eused (PPD, REC and BOP), the histological data that are currently being evaluated might reveal more subtle differences in soft tissue response towards ZrO_2 and Ti abutment surfaces.

Overall, the null hypotheses that permucosal sites adjacent to ZrO_2 and Ti abutment surfaces exhibit more or less similar clinical characteristics of peri-implant health and microbiological features during the first 3 months could not be convincingly rejected for most parameters with the exception of the pocket probing depth. Somewhat shallower probing depths were observed around ZrO_2 abutments after 3 months.

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