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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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Abstract
Aim: To compare the early bacterial colonization and soft tissue health of mucosa adjacent to zirconia (ZrO₂) 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 ZrO₂ or a Ti abutment (non-submerged implant placement, within-subject comparison, left-right randomization). Sucuclar 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 ZrO₂ and Ti abutments, at the two observation time points.

Results: ZrO₂ 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 ZrO₂ and Ti abutments at all times, without significant differences in most clinical parameters of peri-implant tissue health. Mean probing depths around Ti abutments were slightly deeper than around ZrO₂ 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 ZrO₂ and Ti abutment surfaces or in early bacterial colonization could be demonstrated, although somewhat shallower probing depths were observed around ZrO₂ 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 and as copings for ceramic crowns, is increasing. Zirconia (ZrO₂) 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 ZrO₂-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 ZrO₂ and on the biomechanical properties of these materials (Wenz et al. 2008). Much less information is available regarding the soft tissue response to ZrO₂ 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 (Quiryjen et al. 1993, 1994; Bollen et al. 1996a; Rimondini et al. 1997; Abrahamsson et al. 1993, 1994; Bollen et al. 1996b; 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
The potential advantages of ZrO₂ compared with Ti, with respect to biofilm formation in the oral cavity, has been demonstrated in various studies. ZrO₂ 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 ZrO₂, 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 ZrO₂ and Ti in permucosal applications. Less pronounced inflammation-related processes were noticed around ZrO₂ 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 ZrO₂ and Ti abutment surfaces and on early submucosal bacterial colonization. These issues are compared under the null hypotheses that permucosal sites adjacent to ZrO₂ 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 4.0 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 cuspsids. 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) ZrO₂ 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 (ZrO₂ 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 ZrO₂ specimen (Astra Tech AB). Abutment materials and production methods were basically similar to those used in the production of commercially available, regular Ti and ZrO₂ 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 ZrO₂ and Ti abutments was measured at three locations on one specimen of each material by means of contact profilometry. Mean Rₐ-values were 236 nm (range: 217–255 nm) for the ZrO₂ abutment and 210 nm (range: 173–272 nm) for the Ti abutment. The corresponding Rₛ-values were 292 nm (range: 260–330 nm) and 259 nm (range: 220–332 nm) for the ZrO₂ 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 ZrO₂ 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 forsythii (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), 300nM of species-specific primers, 100nM 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, 2003a) and those for T. denticola by Kuboniwa et al. (2004b). Five microliters 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 H2O 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 ZrO2 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 ZrO2 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 ZrO2 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 ZrO2 compared with Ti abutments. No further statistically significant clinical differences between ZrO2, and Ti abutments were observed for meanPPD, meanREC or BOP at 2 weeks or 3 months. The meanPPD decreased significantly for both the ZrO2 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 ZrO2 and Ti abutments, both at 2 weeks and at 3 months. Generally, slightly larger numbers of bacteria were found at the ZrO2 abutment surfaces compared with the Ti surfaces, although this never reached a statistically significant level [Table 2].

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

<table>
<thead>
<tr>
<th></th>
<th>2 weeks</th>
<th>3 months</th>
<th>P-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZrO2</td>
<td>3 (1.1)</td>
<td>1.7 (0.7)</td>
<td>Z_{3.65}, P=0</td>
</tr>
<tr>
<td>Ti</td>
<td>2.9 (0.8)</td>
<td>2.2 (0.8)</td>
<td>Z_{2.16}, P=0.03</td>
</tr>
<tr>
<td>MeanREC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZrO2</td>
<td>2.1 (1.2)</td>
<td>2.7 (0.6)</td>
<td>Z_{2.49}, P=0.01</td>
</tr>
<tr>
<td>Ti</td>
<td>1.9 (1.2)</td>
<td>2.6 (1)</td>
<td>Z_{2.82}, P=0</td>
</tr>
<tr>
<td>BOP</td>
<td>50%</td>
<td>52.6%</td>
<td>Z_{0.25}, P=0.8</td>
</tr>
<tr>
<td>Ti</td>
<td>75%</td>
<td>47.4%</td>
<td>Z_{0.01}, P=0.05</td>
</tr>
</tbody>
</table>

Pairwise comparison of data after 2 weeks and 3 months for zirconia (ZrO2) 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\textsubscript{2}) and titanium (Ti) abutments (n = 20 subjects for the 2 weeks and n = 19 subjects for the 3 month interval)

<table>
<thead>
<tr>
<th>Subject</th>
<th>2 weeks (n = 20)</th>
<th>3 months (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZrO\textsubscript{2}</td>
<td>Ti</td>
</tr>
<tr>
<td>Aa</td>
<td>1 2 0 0 3 1 1 0 7 6 17 15 2 1</td>
<td>19 20 17 20</td>
</tr>
<tr>
<td>Pg</td>
<td>4670 220 0 0</td>
<td>4150 91</td>
</tr>
<tr>
<td>Pi</td>
<td>0 190 0 0</td>
<td>6802 0</td>
</tr>
<tr>
<td>Tf</td>
<td>4670 220 0 0</td>
<td>440 91</td>
</tr>
<tr>
<td>Pm</td>
<td>0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>Fn</td>
<td>0 0 0 0</td>
<td>36,770</td>
</tr>
<tr>
<td>Td</td>
<td>0 0 0 0</td>
<td>1,000,000</td>
</tr>
</tbody>
</table>

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 n = 19 subjects for the 3 month interval)

<table>
<thead>
<tr>
<th>Subject</th>
<th>2 weeks</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZrO\textsubscript{2}</td>
<td>Ti</td>
</tr>
<tr>
<td>1</td>
<td>0 0 1 2</td>
<td>4 5</td>
</tr>
<tr>
<td>2</td>
<td>1700 69,000</td>
<td>1,810,000</td>
</tr>
<tr>
<td>3</td>
<td>298,631 19,390,200</td>
<td>853,000</td>
</tr>
<tr>
<td>4</td>
<td>1540 1249</td>
<td>1,280,000</td>
</tr>
<tr>
<td>5</td>
<td>114 4260</td>
<td>400,019</td>
</tr>
<tr>
<td>6</td>
<td>3006 266</td>
<td>690,620</td>
</tr>
<tr>
<td>7</td>
<td>220,000 220,000</td>
<td>2300</td>
</tr>
<tr>
<td>8</td>
<td>50 2540</td>
<td>0 0</td>
</tr>
<tr>
<td>9</td>
<td>30,410 170</td>
<td>3300</td>
</tr>
<tr>
<td>10</td>
<td>1,804,110 1420</td>
<td>124,200</td>
</tr>
<tr>
<td>11</td>
<td>22,213,000 66,000</td>
<td>4900</td>
</tr>
<tr>
<td>12</td>
<td>3230 290</td>
<td>3400</td>
</tr>
<tr>
<td>13</td>
<td>3100 1540</td>
<td>650,170</td>
</tr>
<tr>
<td>14</td>
<td>650 650</td>
<td>4,800,160</td>
</tr>
<tr>
<td>15</td>
<td>340 400</td>
<td>16,000</td>
</tr>
<tr>
<td>16</td>
<td>0 374</td>
<td>4,530,000</td>
</tr>
<tr>
<td>17</td>
<td>656 970</td>
<td>48,000</td>
</tr>
<tr>
<td>18</td>
<td>43,000 4500</td>
<td>3200</td>
</tr>
<tr>
<td>19</td>
<td>58 0 8600</td>
<td>38,900</td>
</tr>
<tr>
<td>20</td>
<td>750 1,990,041 2,903,700</td>
<td>136,000</td>
</tr>
</tbody>
</table>

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”.

Discussion

ZrO\textsubscript{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\textsubscript{2} abutments and restorations are scarce. The same can be said with respect to the soft tissue response to ZrO\textsubscript{2} itself, because well-controlled in vivo human studies are lacking as was also postulated in a consensus statement on soft tissue integration (Klinge & Meyke 2006). The present study deals with the peri-implant soft tissue response to ZrO\textsubscript{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\textsubscript{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 bacterial colonization are presumably the result of differences in surface-free energy (electrical conductivity). The surface roughness
of the abutments that were used ($R_s$-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 evaluation 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]. Any 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®, Kerr/Hawe, 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. 2003b; 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 cued (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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